

NTIC FILE COPY

20030205089

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION
Unclassified

ELECTE

1b. RESTRICTIVE MARKINGS

2a. SECURITY CLASSIFICATION AUTHORITY

OCT 16 1989

3. DISTRIBUTION/AVAILABILITY OF REPORT
Approved for public release;
distribution unlimited

AD-A213 266

LE Bα D
R(S)

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION
University of South Carolina
School of Medicine

6b. OFFICE SYMBOL
(If applicable)

7a. NAME OF MONITORING ORGANIZATION

6c. ADDRESS (City, State, and ZIP Code)

Columbia, South Carolina 29208

7b. ADDRESS (City, State, and ZIP Code)

8a. NAME OF FUNDING/SPONSORING
ORGANIZATION U.S. Army Medical
Research & Development Command

8b. OFFICE SYMBOL
(If applicable)

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
Contract No. DAMD17-86-C-6118

8c. ADDRESS (City, State, and ZIP Code)

Fort Detrick
Frederick, Maryland 21701

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
61102A	3M1 61102BS12	AD	130

11. TITLE (Include Security Classification)

Biology of Immunomodulators

12. PERSONAL AUTHOR(S)

Abdul Chaffar; J. David Gangemi; Eugene P. Mayer

13a. TYPE OF REPORT
Annual Report

13b. TIME COVERED
FROM 2/15/87 TO 2/14/88

14. DATE OF REPORT (Year, Month, Day)
1988 July

15. PAGE COUNT
109

16. SUPPLEMENTARY NOTATION

17. COSATI CODES

FIELD	GROUP	SUB-GROUP
06	03	
06	16	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

RAI; Immunoenhancing drugs; Viruses; Vaccines.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

A number of pyrimidinones were compared for their effects on the reticuloendothelial system (RES) function macrophage cytotoxicity, prostaglandin secretion, serum interferon levels and resistance to herpes, Aichi and Banz virus infections. Five of these agents (ABPP, ACPP, AIPP, ABMFPP and ACDFFP) stimulated the RES function when tested two days after treatment. However, their effects were not as pronounced four days post treatment. They also caused a reduction in prostaglandin secretion by macrophages when given 2, 4, and 7 days before sampling. ABPP, ACPP and ABMFPP but not AIPP and ACDFFP rendered macrophages cytotoxic. Five of the pyrimidinones tested caused an elevation in serum interferon levels which peaked between one and two days after treatment. However, the magnitude of the response varied with the drug; ACPP and ABMFPP were most effective while AIPP and ABMP were moderately effective and the effect of ABPP was marginal. Pyrimidinones were most effective in enhancing resistance against Banz virus induced encephalitis. Four of the drugs (ACPP, AIPP, ABMFPP and ACDFFP) increased resistance when given prophylactically and three (ABPP, ABMFPP and ACDFFP) when given on the day of challenge. In the herpes virus encephalitis model, only ABPP was effective and only prophylactically. Likewise, only ABMFPP had some effect in the influenza model and only on the day of challenge.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT

☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION
Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL
Mrs. Virginia M. Miller

22b. TELEPHONE (Include Area Code)
301/663-7325

22c. OFFICE SYMBOL
SCRD-FM1-S

89 10 16 069

AD _____

BIOLOGY OF IMMUNOMODULATORS

Annual Report

J. DAVID GANGEMI
ABDUL CHAFFAR
EUGENE P. MAYER

July 1988

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

DAMD17-86-C-6118

University of South Carolina
School of Medicine
Columbia, South Carolina 29208

DOD DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

The Findings in this report are not to be construed
as an official Department of the Army position unless
so designated by other authorized documents

SUMMARY

We have compared effects of a number of different pyrimidinones (ABPP, ACPP, AIPP, ABMP, ABMFPP and ACDFPP) on clearance and organ localization of radiolabelled sheep erythrocytes (SRBC); macrophage cytotoxicity; prostaglandin secretion and serum interferon levels. We have also examined the effect of these agents on resistance to herpes, Aichi and Banzi virus infections.

Five of these agents (ABPP, ACPP, AIPP, ABMFPP and ACDFPP) were examined for their effects on the reticuloendothelial system function and they were all capable of stimulating this function when tested two days after treatment. However these effects were not as pronounced four days after treatment. These pyrimidinones also caused a reduction in prostaglandin secretion by macrophages when given 2, 4 and 7 days before sampling. In addition, three of the pyrimidinones (ABPP, ACPP and ABMFPP) caused activation of macrophages to become cytotoxic whereas the other two (AIPP and ACDFPP) were without effect. Of the five pyrimidinones tested for their effect on serum interferon levels, AIPP and ABMP produced a marginal increase, ABPP a moderate increase and ACPP and ABMFPP a large increase. In all cases the peak response was observed between days 1 and 2 post treatment.

The most beneficial effect of these pyrimidinones was observed in the Banzi virus encephalitis model. Four of the drugs (ACPP, AIPP, ABMFPP and ACDFPP) increased resistance when given prophylactically and three (ABPP, ABMFPP and ACDFPP) when given on the day of challenge. In the herpesvirus encephalitis model, only ABPP affected the resistance and only when given prophylactically. Likewise, only ABMFPP had some effect in the influenza model and only when given on the day of challenge. None of the drugs were effective in the herpesvirus hepatitis model.



Accession For	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978)

TABLE OF CONTENTS

Summary.....	1
Foreword.....	2
Table of Contents.....	3
List of Appendices.....	4
Body of Report	
Problem under Investigation.....	10
Background.....	11
Approach.....	14
Results.....	16
Conclusions.....	19
Recommendations.....	20
Literature Cited.....	21
Appendices	
Tables 1-8.....	24
Figures 1-77.....	32
Distribution List.....	108

LIST OF APPENDICES

Tables

- Table 1. Clearance and tissue localization of SRBC following treatment with various pyrimidinones on day -2.
- Table 2. Clearance and tissue localization of SRBC following treatment with various pyrimidinones on day -4.
- Table 3. Activation of cytotoxic macrophages by pyrimidinones.
- Table 4. PGE-2 secretion by peritoneal macrophages from ABPP treated mice.
- Table 5. PGE-2 secretion by peritoneal macrophages from AIPP treated mice.
- Table 6. PGE-2 secretion by peritoneal macrophages from ACPD treated mice.
- Table 7. PGE-2 secretion by peritoneal macrophages from ABMFPP treated mice.
- Table 8. PGE-2 secretion by peritoneal macrophages from ACDFFPP treated mice.

Figures

- Figure 1. Serum interferon levels following treatment with AIPP.
- Figure 2. Serum interferon levels following treatment with ABMP.
- Figure 3. Serum interferon levels following treatment with ABPP.
- Figure 4. Serum interferon levels following treatment with ACPP.
- Figure 5. Serum interferon levels following treatment with ABMFPP.
- Figure 6. Effect of ABPP, given on day -2, on resistance to influenza-induced pneumonitis.
- Figure 7. Effect of ABPP, given on day 0, on resistance to influenza-induced pneumonitis.
- Figure 8. Effect of ABPP, given on day +1, on resistance to influenza-induced pneumonitis.
- Figure 9. Effect of ACPP, given on day -2, on resistance to influenza-induced pneumonitis.
- Figure 10. Effect of ACPP, given on day 0, on resistance to influenza-induced pneumonitis.
- Figure 11. Effect of ACPP, given on day +1, on resistance to influenza-induced pneumonitis.
- Figure 12. Effect of AIPP, given on day -2, on resistance to influenza-induced pneumonitis.
- Figure 13. Effect of AIPP, given on day 0, on resistance to influenza-induced pneumonitis.
- Figure 14. Effect of AIPP, given on day +1, on resistance to influenza-induced pneumonitis.
- Figure 15. Effect of ABMP, given on day -2, on resistance to influenza-induced pneumonitis.
- Figure 16. Effect of ABMP, given on day 0, on resistance to influenza-induced pneumonitis.
- Figure 17. Effect of ABMP, given on day +1, on resistance to influenza-induced pneumonitis.

- Figure 18. Effect of ABMFPP, given on day -2, on resistance to influenza-induced pneumonitis.
- Figure 19. Effect of ABMFPP, given on day 0, on resistance to influenza-induced pneumonitis.
- Figure 20. Effect of ABMFPP, given on day +1, on resistance to influenza-induced pneumonitis.
- Figure 21. Effect of ACDFPP, given on day -2, on resistance to influenza-induced pneumonitis.
- Figure 22. Effect of ACDFPP, given on day 0, on resistance to influenza-induced pneumonitis.
- Figure 23. Effect of ACDFPP, given on day +1, on resistance to influenza-induced pneumonitis.
- Figure 24. Effect of ABPP, given on day -2, on resistance to herpesvirus-induced hepatitis.
- Figure 25. Effect of ABPP, given on day 0, on resistance to herpesvirus-induced hepatitis.
- Figure 26. Effect of ABPP, given on day +1, on resistance to herpesvirus-induced hepatitis.
- Figure 27. Effect of ACPP, given on day -2, on resistance to herpesvirus-induced hepatitis.
- Figure 28. Effect of ACPP, given on day 0, on resistance to herpesvirus-induced hepatitis.
- Figure 29. Effect of ACPP, given on day +1, on resistance to herpesvirus-induced hepatitis.
- Figure 30. Effect of AIPP, given on day -2, on resistance to herpesvirus-induced hepatitis.
- Figure 31. Effect of AIPP, given on day 0, on resistance to herpesvirus-induced hepatitis.
- Figure 32. Effect of AIPP, given on day +1, on resistance to herpesvirus-induced hepatitis.
- Figure 33. Effect of ABMP, given on day -2, on resistance to herpesvirus-induced hepatitis.
- Figure 34. Effect of ABMP, given on day 0, on resistance to herpesvirus-induced hepatitis.

- Figure 35. Effect of ABMP, given on day +1, on resistance to herpesvirus-induced hepatitis.
- Figure 36. Effect of ABMFPP, given on day -2, on resistance to herpesvirus-induced hepatitis.
- Figure 37. Effect of ABMFPP, given on day 0, on resistance to herpesvirus-induced hepatitis.
- Figure 38. Effect of ABMFPP, given on day +1, on resistance to herpesvirus-induced hepatitis.
- Figure 39. Effect of ACDFPP, given on day -2, on resistance to herpesvirus-induced hepatitis.
- Figure 40. Effect of ACDFPP, given on day 0, on resistance to herpesvirus-induced hepatitis.
- Figure 41. Effect of ACDFPP, given on day +1, on resistance to herpesvirus-induced hepatitis.
- Figure 42. Effect of ABPP, given on day -2, on resistance to herpesvirus-induced encephalitis.
- Figure 43. Effect of ABPP, given on day 0, on resistance to herpesvirus-induced encephalitis.
- Figure 44. Effect of ABPP, given on day +1, on resistance to herpesvirus-induced encephalitis.
- Figure 45. Effect of ACPP, given on day -2, on resistance to herpesvirus-induced encephalitis.
- Figure 46. Effect of ACPP, given on day 0, on resistance to herpesvirus-induced encephalitis.
- Figure 47. Effect of ACPP, given on day +1, on resistance to herpesvirus-induced encephalitis.
- Figure 48. Effect of AIPP, given on day -2, on resistance to herpesvirus-induced encephalitis.
- Figure 49. Effect of AIPP, given on day 0, on resistance to herpesvirus-induced encephalitis.
- Figure 50. Effect of AIPP, given on day +1, on resistance to herpesvirus-induced encephalitis.
- Figure 51. Effect of ABMP, given on day -2, on resistance to herpesvirus-induced encephalitis.

- Figure 52. Effect of ABMP, given on day 0, on resistance to herpesvirus-induced encephalitis.
- Figure 53. Effect of ABMP, given on day +1, on resistance to herpesvirus-induced encephalitis.
- Figure 54. Effect of ABMFPP, given on day -2, on resistance to herpesvirus-induced encephalitis.
- Figure 55. Effect of ABMFPP, given on day 0, on resistance to herpesvirus-induced encephalitis.
- Figure 56. Effect of ABMFPP, given on day +1, on resistance to herpesvirus-induced encephalitis.
- Figure 57. Effect of ACDFPP, given on day -2, on resistance to herpesvirus-induced encephalitis.
- Figure 58. Effect of ACDFPP, given on day 0, on resistance to herpesvirus-induced encephalitis.
- Figure 59. Effect of ACDFPP, given on day +1, on resistance to herpesvirus-induced encephalitis.
- Figure 60. Effect of ABPP, given on day -2, on resistance to banzivirus-induced encephalitis.
- Figure 61. Effect of ABPP, given on day 0, on resistance to banzivirus-induced encephalitis.
- Figure 62. Effect of ABPP, given on day +1, on resistance to banzivirus-induced encephalitis.
- Figure 63. Effect of ACPP, given on day -2, on resistance to banzivirus-induced encephalitis.
- Figure 64. Effect of ACPP, given on day 0, on resistance to banzivirus-induced encephalitis.
- Figure 65. Effect of ACPP, given on day +1, on resistance to banzivirus-induced encephalitis.
- Figure 66. Effect of AIPP, given on day -2, on resistance to banzivirus-induced encephalitis.
- Figure 67. Effect of AIPP, given on day 0, on resistance to banzivirus-induced encephalitis.
- Figure 68. Effect of AIPP, given on day +1, on resistance to banzivirus-induced encephalitis.

- Figure 69. Effect of ABMP, given on day -2, on resistance to
banzivirus-induced encephalitis.
- Figure 70. Effect of ABMP, given on day 0, on resistance to
banzivirus-induced encephalitis.
- Figure 71. Effect of ABMP, given on day +1, on resistance to
banzivirus-induced encephalitis.
- Figure 72. Effect of ABMFPP, given on day -2, on resistance to
banzivirus-induced encephalitis.
- Figure 73. Effect of ABMFPP, given on day 0, on resistance to
banzivirus-induced encephalitis.
- Figure 74. Effect of ABMFPP, given on day +1, on resistance to
banzivirus-induced encephalitis.
- Figure 75. Effect of ACDFPP, given on day -2, on resistance to
banzivirus-induced encephalitis.
- Figure 76. Effect of ACDFPP, given on day 0, on resistance to
banzivirus-induced encephalitis.
- Figure 77. Effect of ACDFPP, given on day +1, on resistance to
banzivirus-induced encephalitis.

I. PROBLEM UNDER INVESTIGATION

This study was designed to evaluate the multifaceted effects of selected immunoenhancing drugs on specific and nonspecific components of the immune system which are of importance in resistance to and recovery from viral infections. We have examined the effect of treatment schedule on various in vitro and in vivo immune parameters. The immune parameters examined included:

- A. In Vitro / Ex Vivo Evaluation of Nonspecific Elements Affecting the Course of Viral Disease:
 - 1. Macrophage antiviral cytotoxicity
 - 2. Natural killer (NK) cell cytotoxicity
 - 3. Production of interferons (IF)
 - 4. Clearance of radiolabeled erythrocytes from blood and their localization in various organs
 - 5. Phagocytosis by peritoneal, splenic and liver macrophages
- B. In vitro / Ex Vivo Evaluation of Specific Elements Affecting Resistance to and Recovery from Viral Diseases:
 - 1. Antibody responses to T-dependent antigens
 - 2. T cell cytotoxicity
 - 3. Alterations in T and B lymphocyte populations and subpopulations (e.g., T helper or suppressor cells)
- C. Evaluation of Host Resistance to and Recovery from Viral Infections:

II. BACKGROUND

Members of the military are exposed to a variety of viruses which often result in infections leading to serious illness or death. Although they can sometimes be protected by active immunization, this approach is not always practical due to difficulties in producing either attenuated or killed vaccines which are both safe and immunogenic. In addition, vaccines are of little value in the therapy of active viral infections. Therefore, alternative approaches have been explored. One approach has been the development of antiviral drugs. While these drugs have been effective, in some situations, their use has been hampered by their toxic side effects and limited range of activity.

Another approach to prevention and treatment of viral infections has been immunotherapy. Although immunotherapy with classical agents has had some success, it has also been plagued by toxicity problems. However, the recent development of chemically defined or synthetic immunostimulants with low toxicity and broad spectrum activity has made this approach more appealing. These immunostimulants have been used alone and in combination with vaccines in prophylaxis or with antiviral compounds in therapy.

While there are numerous reports of the efficacy of the newer generation immunostimulants, the experimental approaches utilizing these compounds have varied, thus, making an objective analysis of their comparative efficacy difficult. In addition, since the cellular components of the immune system that need to be stimulated will vary depending on the pathogenic features of the virus, it is essential that the mode of action of immunostimulating drugs be defined. Because the comparative efficacy and mode of action of many immunostimulants have not been fully explored their use has been mostly empirical. A more rational approach for the selection of appropriate drugs for use in prophylaxis or therapy requires 1) a comparison of the efficacy of various agents under the same experimental conditions and with the same panel of tests and 2) a better understanding of their modes of action.

Most immunostimulants possess a unique set of immunomodulating features and provide varying degrees of benefit to the infected host. The beneficial effects imparted by these immunostimulants will largely depend on the tissue site and degree of virus infection. For example, it may be desirable to have elevated levels of interferon in some tissue sites during a particular time of infection but not during others. This may be particularly relevant in some arenavirus infections (e.g., lymphocytic choriomeningitis virus; LCMV) in which interferon can have detrimental effects (1, 2). Likewise, activated NK cells and macrophages may result in immunopathologic damage which can contribute to the disease process (3). Because of these complexities, the choice of immunomodulating agents, their dose, time

and frequency of administration require careful consideration of the immunopathologic features of infection. This is only possible if one is able to identify the spectrum of changes induced by a particular drug.

By virtue of their position at sites of initial infection and wide distribution in major organs of the body, macrophages and NK cells and their soluble mediators (e.g., PG, IL, MAF and IF) are thought to be of prime importance in resistance to a number of intracellular pathogens. Thus, for many viral infections macrophage function has been shown to be an important factor in determining the course of the disease (4-7). For example, in herpesvirus infections both resistance to virus replication within macrophages (intrinsic resistance) and macrophage antiviral effects on other virus infected cells (extrinsic antiviral activity) may be significant determinants in host resistance. (8)

In addition to macrophages, another cell type which plays a significant role in primary resistance to virus infection is the NK cell (9-11). Unlike the cytotoxic T lymphocyte, this cell destroys virus infected cells without prior sensitization and thus quickly limits virus dissemination (11). A positive correlation between genetically determined resistance to virus lethality and the level of NK cell augmentation has been observed in both murine cytomegalovirus and herpes simplex virus infections (12,13).

A variety of soluble mediators may be released following the administration of various immunostimulants. Some of these mediators may have a negative effect on the immune system while others may have a positive effect. For example, prostaglandins may have a detrimental effect due to their negative feedback control on cellular functions (14-16). In contrast, interferon has a beneficial role in inhibition of virus replication as well as in the augmentation of cellular components of the immune system. While each type of interferon (i.e. alpha, beta and gamma) possess the ability to induce the antiviral state in cells, gamma interferon may be more important since it also regulates various immune functions (17-19).

There are a number of reports on the use of macrophage activators in the treatment of infectious diseases. Most notably, these compounds have been used prophylactically to enhance nonspecific resistance by direct activation of macrophages and NK cells or via the induction of soluble mediators. For example, inoculation of mice with Escherichia coli endotoxin, Staphylococcus aureus, BCG, or the lipoidal amine (CP-20,961) enhances resistance to influenza virus through the induction of interferon and/or the activation of macrophages and NK cells (20-23). Similar effects against herpesviruses, Newcastle disease, encephalomyocarditis, vesicular stomatitis, and Junin viruses were observed after treatment with various immunostimulants (24-30). Likewise, inoculation of mice with E. agnes induced protection against various hemoprotozoans (31-34).

In addition to their effects on macrophages and NK cells immunostimulating agents also affect elements of the specific immune response. Since both antibody and cell mediated immune responses are involved in resistance to and recovery from viral infections, immunostimulating drugs have been used in combination with whole, and subunit viral vaccines in an attempt to enhance their immunogenicity (35, 36). Use of immunostimulants may be particularly valuable in those situation in which cloned vaccines are available, since these antigens are poor immunogens.

Unfortunately, selection of appropriate immunostimulants to use with vaccines has been somewhat empirical. This is due to the variety of cellular targets on which immunostimulants can act, and the paucity of information concerning the their effects on these targets. For example, some immunostimulants, or the soluble mediators released in response to them, may selectively potentiate B cells, or suppressor or helper T cells which may influence the quantity of antibody produced following vaccination (37-39). In contrast, other immunostimulants may preferentially augment cytotoxic T cells which can have profound effects on recovery from viral disease but have little impact on resistance to viral infection.

In summary, immunoenhancing drugs can exert their effect by interacting with one or more of the cellular components of the immune system. These components are affected either directly, or indirectly through the action of soluble mediators. The ultimate outcome of such drug interactions will depend upon which of the various components is influenced. Therefore, the judicious use of immunoenhancing drugs, together with vaccines in prophylaxis or in the therapy of viral infections of military importance, requires a thorough understanding of their relative effects on the numerous components of the immune system.

While the prophylactic use of immunopotentiating substances has been widely studied, their therapeutic value has not been well documented. In addition, the comparative efficacy and mode of action of various immunostimulants against a variety of infectious agents (especially those of military significance) has not been adequately examined.

Our studies will provide the comparative data on a spectrum of immunological parameters for various immunoenhancing drugs. These data will provide a more scientific basis for the use of various immunoenhancing agents, either alone or in combination with vaccines or antivirals, in the effective treatment of viral diseases of importance to the military.

III. EXPERIMENTAL APPROACH

In this project each immunoenhancing drug was studied in two phases. During the first phase we examined the effects of selected drugs on a variety of components in the immune system. In the second phase we applied the knowledge gained from the initial phase to design experimental protocols to evaluate the clinical potential of these drugs. The studies were performed in animal models of human viral disease.

Phase I consisted of experiments designed to characterize the effects which selected immunostimulants exerted on the nonspecific or specific components of the immune system. Drugs were administered to C3H/HeN mice, intraperitoneally (i.p.), intravenously (iv.) or orally and appropriate cells or fluids obtained at selected intervals. The cells were examined in vitro for a variety of effector functions and their characteristic surface markers. The fluids were examined for the presence of soluble mediators. The effects of time of treatment was also assessed.

Phase II studies were designed to assess the effects of immunostimulants on resistance to and recovery from viral infection. Based on the immunological profiles from phase I and the pathogenesis of the viral agents under study, appropriate drugs were selected for either prophylaxis or therapy. Animals were examined for their ability to survive challenge with lethal doses of infectious agent. These experiments were performed using murine models of influenza virus, herpesvirus, and Banzai virus infections. Lung, liver and brain infections were studied. The following animal models were employed.

Influenza Virus Pneumonitis: The virus used in these studies is a mouse adapted H3N2 strain of influenza A virus (Aichi). When 2-10 LD₅₀ of this strain is administered intranasally into six to seven week old C3H/HeN mice, death, due to interstitial pneumonia, occurs in five to seven days. Virus is found only in the lungs and mice eventually die of pneumonia.

HSV-1 Encephalitis: The virus used to induce encephalitis is a human isolate (MB strain) of type 1 herpes simplex virus obtained from Dr. Richard Whitley (Univ. Ala, Birmingham, AL). Footpad inoculation of four week old C3H/HeN mice results in virus replication in the sciatic nerve, spinal cord and brain. Mice die of encephalitis six to eight days after inoculation. Immunoperoxidase staining for viral antigen has been used to confirm this mode of virus dissemination.

HSV-1 Hepatitis: The MB virus strain was used to induce liver disease. When four to five week old C3H/HeN mice are inoculated intravenously with 2-10 LD₅₀ of virus, the primary organ of initial infection is the liver. Viremia and dissemination to a number of

other organs follows liver infection and death results five to seven days post infection.

Banzi Virus Encephalitis: The seed virus used in these studies was obtained from Dr. C.J. Peters (USAMRIID, Fort Detrick, MD). Working stocks of virus are prepared from suckling mouse brains. When inoculated subcutaneously, this virus replicates in peripheral lymphoid tissue and is carried to the spleen. Viremia results 2-4 days post infection and the virus enters the brain. Encephalitis is observed 6-8 days post infection. Death ensues 8-10 days following the administration of as little as 10 p.f.u.

IV. RESULTS

During the second year of this contract, we have focused our studies primarily on the comparative effects of various pyrimidinones on a number of immunological parameters, although, we have also begun investigations on other drugs. The parameters examined included: in vivo clearance and organ localization of radiolabelled sheep erythrocytes (SRBC); peritoneal and splenic cell phagocytosis and activation of cytotoxic macrophages. We have also examined the effect of these agents on resistance to viral models of pneumonitis, hepatitis and encephalitis.

In Vivo Clearance and Organ Localization of Erythrocytes

Tables 1-2 contain data on the effect of the various pyrimidinones on clearance rate of SRBC from circulation and their localization in liver, spleen and lung. The clearance rates are presented as T/2 and K-values. An increase in K-value reflects an increase in the rate of clearance and consequently a decrease in the half-life ($T_{1/2}$) of SRBC in circulation. Also listed in the tables are alpha values which represent clearance rates normalized for mouse body, spleen and liver weights. Thus, increased alpha values also represent increased clearance rates. Organ localization is presented as number of SRBC per mg wet tissue.

Two days after intraperitoneal (ip) administration of all pyrimidinones tested caused an increase in the clearance rate of SRBC which did not appear to be due to alterations in the body or organ weights as indicated by the increase in alpha values. These effects, with the exception of ACPP, were statistically significant (Table 1). This increase was apparently due to increased localization in liver which is the major organ for clearance of particulate material from circulation. Since all pyrimidinones were administered in carboxy methyl cellulose (CMC), it was also necessary to compare the CMC treated group with a saline control group. Such a comparison revealed that CMC itself stimulated the reticuloendothelial functions. Consequently, the effect of drug-carrier mixture was more pronounced when compared with the saline control.

The effects of pyrimidinones were less pronounced when drugs were administered four days before assay as compared to the CMC group. However, these effects were still mostly significant when compared with the saline control (Table 2).

Macrophage Cytotoxicity

Macrophage cytotoxicity was tested by incubating peritoneal adherent cells with virally transformed EL-4 cells for 48 hours and measuring

the incorporation of ^3H -thymidine by the target cells. In these experiments macrophages were harvested four days after ip injection of drugs. Results summarized in table 3 indicate that treatment with ABPP, ACP and ABMFPP caused activation of macrophages to become cytotoxic when compared with macrophages from CMC-treated controls. In this assay, CMC treatment was without effect (compared with the saline control). The cytotoxicity was significant at all effector to target ratios ranging from 40:1 to 10:1. In contrast, AIPP and ACDFPP had no significant effect.

Prostaglandin Secretion by Macrophage

Mice were treated with CMC or pyrimidinones in CMC and peritoneal cells were harvested 2, 4, 7 or 14 days later. Adherent cells were cultured for 20 hours and prostaglandin E-2 levels in supernatants were measured by radioimmunoassay. The results have been summarized in tables 4-8. It is clear that all pyrimidinones caused reduction in prostaglandin secretion by macrophages when given 2, 4 or 7 days before sampling. When macrophages were harvested 14 days after treatment, this effect was variable.

Interferon Levels

Serum interferon levels following treatment with the various pyrimidinones were examined on days, 1, 2, 3, 4 and 7 following drug administration using a VSV plaque reduction assay. These data are summarized in Figures 1-5. Although all pyrimidinones tested caused a noticeable elevation in serum interferon level, the magnitude of the response varied with each drugs. A marginal increase, which was only slightly above the CMC control, was observed with AIPP and ABMP (Figures 1 and 2). ABPP on the other hand produced a moderate increase (Figure 3) and ACP and ABMFPP had a more dramatic effects (Figures 4 and 5). In all case the peak elevation was observed between 1 and 2 days post treatment and was back to background levels by days 3 and 7.

Resistance to Herpes, Influenza and Banzai Virus Infections

The ability of different pyrimidinones to enhance antiviral resistance was examined in murine models of pneumonitis, hepatitis and encephalitis. The results of these experiments are presented below.

Pneumonitis Models

Influenza virus (Aichi strain) was used to induce pneumonitis. In this model 10 LD₅₀ of virus was administered intranasally and mortality monitored for 21 days. Data from these experiments are summarized in Figures 6-23. ABMFPP, when given on the day of

challenge, had a slight, although statistically significant, effect on the mean survival time of infected animals. This treatment also afforded some protection against the infection as 2/10 mice survived until the termination of the experiment (day 21 post-infection) (Figure 19). However, ABMFPP was without effect when given 2 days before, or one day after virus challenge (Figures 18 and 20 respectively). All other pyrimidinones, whether given two days before, on the day of, or one day after challenge, were ineffective.

Hepatitis Models

Herpesvirus (MB-strain) was used to induce hepatitis. In this model 10 LD₅₀ of virus was administered by the iv. route and morbidity and mortality monitored for 21 days. None of the pyrimidinones offered any protection in this model whether given 2 days before, on the day of, or one day after virus infection (Figures 24-41).

Encephalitis Models

Two models of encephalitis were employed, one which uses HSV-1 (given via the foot pad) and the other which uses Banzi virus (given ip.). Data from the herpesvirus experiments are summarized in Figures 42-59. ABPP, when given 2 days before challenge, had a slight, although statistically significant, effect on the mean survival time of infected animals. ABPP also afforded some protection against the infection as 2/10 mice survived until the termination of the experiment (day 21 post-infection) (Figure 42). However, this drug was without effect when given on the day of, or one day after virus challenge (Figures 43 and 44). All other pyrimidinones, whether given two days before, on the day of, or one day after challenge, were ineffective.

The effects of different pyrimidinones on Banzi virus induced encephalitis are summarized in Figures 60-77. When given 2 days before infection, ACPP, AIPP, ABMFPP and ACDFPP had some beneficial effects in prolonging the mean survival time (Figures 63, 66, 72, 75). Three of these also afforded some protection: 1/10 with AIPP (Figure 66) and 2/10 with ABMFPP or ACDFPP (Figures 72 and 75). When given on the day of challenge, ABPP, ABMFPP and ACDFPP prolonged the mean survival time, although none of these drugs afforded any protection (Figures 61, 73, 76). None of the drugs conferred resistance to this virus when given one day after the infection (Figures 62, 65, 68, 71, 74 and 77).

V. CONCLUSIONS

The generated in the second year of this study has resulted in the following conclusions:

1. All pyrimidinones had some RES stimulatory effect.
2. Three of the pyrimidinones cause activation of macrophages to become cytotoxic.
3. All pyrimidinones cause a reduction in prostaglandin secretion.
4. All pyrimidinones cause some increase in serum interferon levels.
5. Only ABMFPP had some protective effect against influenza.
6. None of the pyrimidinones conferred resistance against hepatitis.
7. Only ABPP had slight protective effect against herpesvirus induced encephalitis by several of the pyrimidinones increased resistance against Banzi virus induced encephalitis.

VI. RECOMMENDATIONS

In the forthcoming year we will complete profile on the pyrimidinones, as planned. We will also test the effect of pyrimidinones in selected virus models using a lower challenge dose. In addition we will continue building a profile on new agents which we have recently received.

LITERATURE CITED

1. Trouet, A., Masquelier, M., Baurain, R. and Deprez-De Campeneere, D. Proc. Natl. Acad. Sci. USA 79:626, 1982.
2. Poste, G. and Papahadjopoulos, D. Proc. Natl. Acad. Sci. USA 73:1603, 1976.
3. Alving, C.R. In Targeting of Drugs (G. Gregoriadis, J. Senior and A. Trouet, eds) p 337, Plenum, New York, 1982.
4. Koff, W.C., Showalter, S.D., Hampar, B. and Fidler, I.J. Science 228:495, 1984.
5. Gangemi, J.D., Nachtigal, M., Barnhart, D., Krech, L. and Jani, P. J. Infect. Dis. 155:510, 1987.
6. Bangham, A.D., Standish, M.M. and Watkins, J.C. J. Mol. Biol. 13:238, 1965.
7. Papahadjopoulos, D., Miller, N. Biochem. Biophys. Acta 135:624, 1967.
8. Airian, G., Huang, L. Biophys. J. 25:A292, 1979.
9. Szoka, Jr., F. and Papahadjopoulos, D. Proc. Natl. Acad. Sci. 75:4194, 1978.
10. Fidler, I.J., Raz, A., Fogler, W.E., Kirsh, R., Bugelski, P. and Poste, G. Cancer Res. 40:4460, 1980.
11. Fidler, I.J., Barnes, Z., Fogler, W.E., Kirsh, R., Bugelski, P. and Poste, G. Cancer Res. 42:496, 1982.
12. Rahman, Y.E., Cerny, E.A., Patel, D.R., Lau, E.H. and Wright, B.J. Life Sciences 31:2061, 1982.
13. Ruebush, M.J., Halc, A.H. and Harris, D.T. Infect. Immun. 32:513, 1981.
14. Kramp, W.J., Six, H.B., Drake, S. and Kasel, J.A. Infec. Immun. 25:771, 1979.
15. Neurath, A.R., Kent, S.B.H. and Strick, N. J. Gen. Virol. 65:1009, 1984.
16. Smolin, G., Okumoto, M., Feiler, S., and Condon, D. Amer. J. Ophthal. 91:220, 1981.

17. Kende, M. Alving, C.R., Rill, W., Swartz, G.M. and Canonico, P. Antimicrob. Agents and Chemo. 27:903, 1985.
18. Fidler, I.J., Sone, S., Fogler, W.E. and Barnes, Z.L. Proc. Natl. Acad. Sci. USA 78:1680, 1981.
19. Sone, D. and Fidler, I.J. Cellular Immunol. 57:42, 1981.
20. Lynch, W.E., Sartiano, G.P. and Ghaffar, A. Amer. J. Hematol. 9:249, 1980.
21. Fiume, L., Busi, C. and Mattioli, A. FEBS Lett. 153:6, 1983.
22. Fiume, L., Mattiolo, A., Balboni, P.G., Tognon, M., Barbanti-Brodano, G., De-Vries, J. and Wieland, T. FEBS Lett. 103:47, 1979.
23. Fiume, L., Busi, C., Mattioli, A., Balboni, P.G., Barbanti-Brodano, G. and Wieland, T. In Targeting of Drugs (G. Gregoriadis, Sr., J. and A. Trouet, eds.), pl, Plenum Publ. Co., New York, 1982
24. Fiume, L., Busi, C., Mattioli, A., Balboni, P.G. and Barbanti-Brodano, G. FEBS Lett. 129:261, 1981.
25. Fiume, L., Busi, C. and Mattioli, A. FEBS Lett. 146:42, 1982.
26. Monsigny, M., Roche, A-C. and Midoux, P. Biol. Cell 51:187, 1984.
27. Monsigny, M., Keida, C., Roche, A-C. and Delmotte, F. FEBS Lett. 119:181, 1980.
28. Trouet, A., Baurain, R., Deprez-De Campeneere, D., Masquelier, M. and Prison, P. In Targeting of Drugs (G. Greroriadis, Sr., J. and A. Trouet, eds), pl9, Plenum Publ. Co., New York, 1982.
29. Fiume, L., Mattioli, A., Busi, C., Spinosa, G. and Wieland, T. Experientia 38:1087, 1982.
30. Balboni, P.G., Minia, A., Grossi, M.P., Barbanti-Brodano, G., Mattioli, A. and Fiume, L. Nature 264:181, 1976.
31. Monsigny, M., Roche, A-C. and Bailly, P. Biochem. Biophys. Res. Commun. 121:579, 1984.
32. Roche, A-C., Bailly, P. and Monsigny, M. Invasion Metastasis 5:218, 1985.

33. Galasso, G.J., Merigan, T.C. and Buchanan, R.A. In Antiviral Agents and Viral Diseases of Man, (G.J. Galasso, T.C. Merrigan and R.A. Buchanan, eds), p542, Raven Press, New York, 1984.
34. Ayisi, N.K., Gupta, V.S., Meldrum, J.B., Taneja, A.K. and Babuik, L.A. Antimicrob. Agents Chemother. 17:558, 1980.
35. Fischer, P.H., Lee, J.J., Chen, M.S., Lin, T-S. and Prusoff, W.H. Biochem. Pharmacol. 28:3483, 1979.
36. Hayden, F.G., Douglas, R.G. and Simons, R. Antimicrob. Agents Chemother. 18:536, 1980.
37. Canonico, P.G. In Antibiotics (F.E. Hahn, ed), p161, Springer Verlag Publ. Co., 1980.
38. Trouet, A., Deprez-De Campeneere, D. and de Duve, C. Nature (London) New Biol. 239:110, 1972.
39. Shannon, W.M. and Schabel, F.M. Jr. Pharmacol. Ther. 11:263, 1980.

Table 1. Clearance and tissue localization of SRBC following treatment with various pyrimidinones on day -2.

Treatment		RBC/mg Tissue (x1000)			Phagocytic Index		
		Spleen	Liver	Lung	T/2 (min)	alpha Value	K Value
CMC Control	Mean	148	81	20	3.78	7.32	.0913
	Std. Dev.	24	15	12	1.31	1.28	.0294
ABPP	Mean	103	90	7	1.88	7.86	.1635
	Std. Dev.	37	16	4	.30	.73	.0242
	P-Value	<0.001	NS	<0.001	<0.001	NS	<0.001
ACPP	Mean	100	93	8	3.14	7.33	.1153
	Std. Dev.	30	26	7	1.78	1.46	.0433
	P-Value	<0.001	NS	<0.02	NS	NS	NS
AIPP	Mean	105	104	15	2.28	8.14	.1399
	Std. Dev.	40	15	8	.60	.93	.0325
	P-Value	<0.001	<0.001	NS	<0.005	NS	<0.001
ABMFPP	Mean	63	105	5	2.13	7.51	.1542
	Std. Dev.	23	22	3	.71	.70	.0435
	P-Value	<0.001	<0.005	<0.005	<0.005	NS	<0.001
ACDFPP	Mean	98	87	16	2.66	8.28	.1235
	Std. Dev.	33	25	12	.94	.66	.0338
	P-Value	<0.001	NS	NS	<0.05	<0.05	<0.01
Saline	Mean	149	74	24	4.82	6.47	.0707
	Std. Dev.	43	14	13	1.81	.81	.0257
	P-Value	NS	NS	NS	<0.01	<0.005	<0.01

Pyrimidinones (250 mg/kg) were given intraperitoneally in 1% carboxymethyl-cellulose (CMC) two days before assay. All results are compared with those obtained with the CMC control. A saline control group was also included.

Table 2. Clearance and tissue localization of SRBC following treatment with various pyrimidinones on day -4.

Treatment		RBC/ μ g Tissue ($\times 1000$)			Phagocytic Index		
		Spleen	Liver	Lung	T/2 (min)	alpha Value	K Value
CMC Control	Mean	124	97	20	2.96	7.51	.1129
	Std. Dev.	45	31	28	1.15	.96	.0336
ABPP	Mean	116	85	10	2.00	7.86	.1528
	Std. Dev.	40	11	6	.31	.74	.0193
	P-Value	NS	NS	NS	<0.02	NS	<0.005
ACPP	Mean	114	99	8	2.45	7.57	.1300
	Std. Dev.	47	16	5	.63	.90	.0314
	P-Value	NS	NS	NS	NS	NS	NS
AIPP	Mean	75	109	8	2.25	7.91	.1411
	Std. Dev.	31	23	4	.60	.68	.0328
	P-Value	<0.01	NS	NS	NS	NS	<0.05
ABMFPP	Mean	95	103	6	2.28	7.25	.1079
	Std. Dev.	55	20	5	.85	.59	.0458
	P-Value	<0.05	NS	<0.05	<0.05	NS	<0.005
ACDFPP	Mean	89	102	19	2.48	7.67	.1286
	Std. Dev.	43	14	15	.62	.94	.0328
	P-Value	<0.05	NS	NS	NS	NS	NS
Saline	Mean	148	83	20	3.94	6.82	.0841
	Std. Dev.	60	21	11	1.35	.72	.0276
	P-Value	NS	<0.05	NS	<0.005	<0.005	<0.001

Pyrimidinones (250 mg/kg) were given intraperitoneally in 1% carboxymethyl-cellulose (CMC) 4 days before assay. All results are compared with those obtained with the CMC control. A saline control group was also included.

Table 3. Activation of cytotoxic macrophages by pyrimidinones.

Treatment	PERCENT CYTOTOXICITY					
	Experiment No. 1			Experiment No. 2		
	40:1	20:1	10:1	40:1	20:1	10:1
ABPP	88#	65*	38	99@	96@	51*
ACPP	97#	53	-28	65#	55@	58*
AIPP	21	31	31	21	34	31
ABMFPP	74#	59@	60*	86	31	-42
ACDFPP	19	5	8	17	31	20

Pyrimidinones (250 mg/kg) were given intraperitoneally in 1% carboxymethyl-cellulose (CMC) 4 days before assay. Control mice were give CMC alone. Percent cytotoxicity was calculated as follows:

$$\frac{C-T}{T} \times 100$$

Where, C= Counts per Minute (CPM) in cultures with macrophages from CMC treated mice and T= CPM in cultures from pyrimidinone treated mice.

* p < 0.05

p < 0.01

@ p < 0.001

Table 4. PGE-2 secretion by peritoneal macrophages from ABPP treated mice.

Treatment	pg Prostaglandin E-2 per mg Protein			
	Experiment No.1		Experiment No 2	
	Mean (S.D.)	p	Mean (S.D.)	p
CMC Control	369.2 (12.2)	-	214.5 (49.3)	-
ABPP Day -2	45.3 (2.3)	<0.001	30.2 (11.7)	<0.005
CMC Control	109.0 (27.7)	-	126.6 (20.8)	-
ABPP Day -4	24.1 (2.6)	<0.01	37.1 (11.9)	<0.005
CMC Control	76.9 (3.3)	-	73.4 (4.0)	-
ABPP Day -7	8.3 (1.9)	<0.001	12.2 (2.5)	<0.001
CMC Control	51.1 (14.2)	-	221.7 (73.2)	-
ABPP Day -14	62.1 (46.2)	NS	123.7 (78.1)	NS

Mice were treated intraperitoneally with ABPP (250 mg/kg) in 1% carboxy methyl cellulose (CMC), on various days before assaying for the secretion of PGE-2 by adherent peritoneal exudate cells over a 20 hour time period. PGE-2 levels were determined by radioimmunoassay. Protein content of the adherent cells was determined after lysing a duplicate sample of adherent cells by freezing and thawing three times.

NS = Not significant
ND = Not Done

Table 5. PGE-2 secretion by peritoneal macrophages from ACPD treated mice.

Treatment	pg Prostaglandin E-2 per mg Protein			
	Experiment No.1		Experiment No 2	
	Mean (S.D.)	p	Mean (S.D.)	p
CMC Control	215.0 (55.1)	-	415.1 (104.2)	NS
ACPD Day -2	26.9 (18.8)	<0.02	25.9 (8.9)	<0.005
CMC Control	50.9 (7.0)	-	23.4 (3.3)	-
ACPD Day -4	8.9 (4.2)	<0.001	13.3 (2.1)	<0.02
CMC Control	81.9 (43.0)	-	40.2 (15.7)	-
ACPD Day -7	16.9 (4.7)	<0.05	7.8 (2.0)	<0.025
CMC Control	43.9 (6.7)	-	51.1 (14.2)	-
ACPD Day -14	8.5 (1.3)	<0.001	51.3 (45.4)	NS

Mice were treated intraperitoneally with ACPD (250 mg/kg) in 1% carboxy methyl cellulose (CMC), on various days before assaying for the secretion of PGE-2 by adherent peritoneal exudate cells over a 20 hour time period. PGE-2 levels were determined by radioimmunoassay. Protein content of the adherent cells was determined after lysing a duplicate sample of adherent cells by freezing and thawing three times.

NS = Not significant
ND = Not done

Table 6. PGE-2 secretion by peritoneal macrophages from AIPP treated mice.

Treatment	pg Prostaglandin E-2 per mg Protein			
	Experiment No.1		Experiment No 2	
	Mean (S.D.)	p	Mean (S.D.)	p
CMC Control	283.2 (46.6)	-	215.0 (55.1)	-
AIPP Day -2	27.2 (11.1)	<0.001	39.0 (16.1)	<0.02
CMC Control	50.9 (7.0)	-	23.4 (3.3)	-
AIPP Day -4	8.2 (2.1)	<0.001	9.0 (2.9)	<0.005
CMC Control	81.9 (43.0)	-	73.4 (4.0)	-
AIPP Day -7	11.7 (2.1)	<0.05	11.7 (1.4)	<0.001
CMC Control	43.9 (6.7)	-	51.1 (14.2)	-
AIPP Day -14	10.1 (1.9)	<0.005	8.0 (3.1)	<0.05

Mice were treated intraperitoneally with AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), on various days before assaying for the secretion of PGE-2 by adherent peritoneal exudate cells over a 20 hour time period. PGE-2 levels were determined by radioimmunoassay. Protein content of the adherent cells was determined after lysing a duplicate sample of adherent cells by freezing and thawing three times.

NS = Not significant

Table 7. PGE-2 secretion by peritoneal macrophages from ABMFPP treated mice.

Treatment	pg Prostaglandin E-2 per mg Protein			
	Experiment No.1		Experiment No 2	
	Mean (S.D.)	p	Mean (S.D.)	p
CMC Control	81.7 (31.1)	-	106.4 (27.0)	-
ABMFPP Day -2	18.2 (17.3)	<0.05	27.2 (2.7)	<0.01
CMC Control	50.9 (7.0)	-	23.4 (3.3)	-
ABMFPP Day -4	9.5 (3.4)	<0.001	7.6 (0.4)	<0.005
CMC Control	81.9 (43.0)	-	40.2 (15.7)	-
ABMFPP Day -7	8.5 (0.8)	<0.05	4.1 (0.5)	<0.02
CMC Control	43.9 (6.7)	-	51.1 (14.2)	-
ABMFPP Day -14	90.0 (45.0)	<0.02	8.1 (2.4)	<0.01

Mice were treated intraperitoneally with ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), on various days before assaying for the secretion of PGE-2 by adherent peritoneal exudate cells over a 20 hour time period. PGE-2 levels were determined by radioimmunoassay. Protein content of the adherent cells was determined after lysing a duplicate sample of adherent cells by freezing and thawing three times.

NS = Not significant

Table 8. PGE-2 secretion by peritoneal macrophages from ACDFFP treated mice.

Treatment	pg Prostaglandin E-2 per mg Protein			
	Experiment No.1		Experiment No 2	
	Mean (S.D.)	p	Mean (S.D.)	p
CMC Control	81.7 (31.1)	-	106.4 (27.0)	-
ACDFPP Day -2	9.5 (2.4)	<0.02	31.6 (3.5)	<0.01
CMC Control	50.9 (7.0)	-	23.4 (3.3)	-
ACDFPP Day -4	6.1 (1.9)	<0.001	10.7 (2.1)	<0.005
CMC Control	81.9 (43.0)	-	73.4 (4.0)	-
ACDFPP Day -7	12.0 (4.4)	<0.05	5.8 (2.1)	<0.001
CMC Control	43.9 (6.7)	-	51.1 (14.2)	-
ACDFPP Day -14	76.7 (20.5)	NS	4.4 (1.5)	<0.005

Mice were treated intraperitoneally with ACDFFP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), on various days before assaying for the secretion of PGE-2 by adherent peritoneal exudate cells over a 20 hour time period. PGE-2 levels were determined by radioimmunoassay. Protein content of the adherent cells was determined after lysing a duplicate sample of adherent cells by freezing and thawing three times.

NS = Not significant

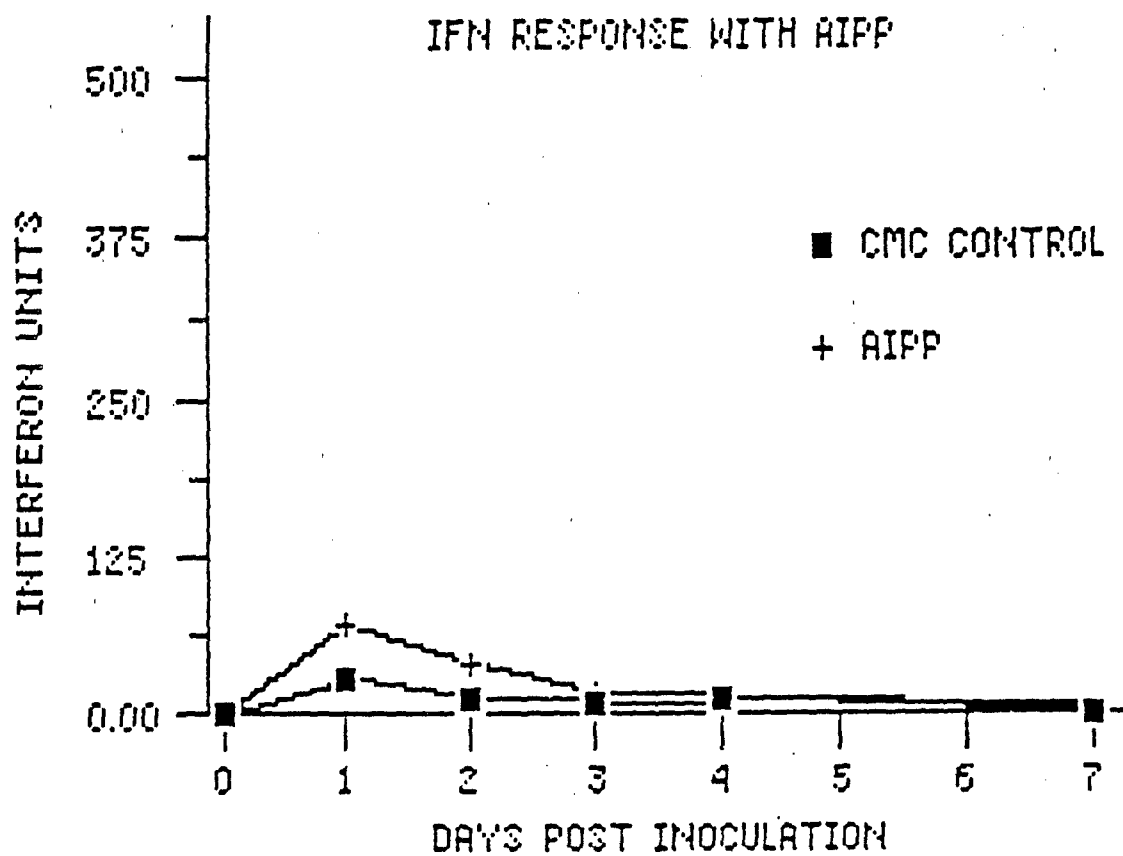


Figure 1. Serum interferon levels following treatment with AIPP. Mice were injected ip with 0.2ml CMC or 250 mg/kg drug in 0.2 ml CMC and then bled on days indicated. Day 0 bleed was obtained immediately before injection. Each point represents a mean of three mice.

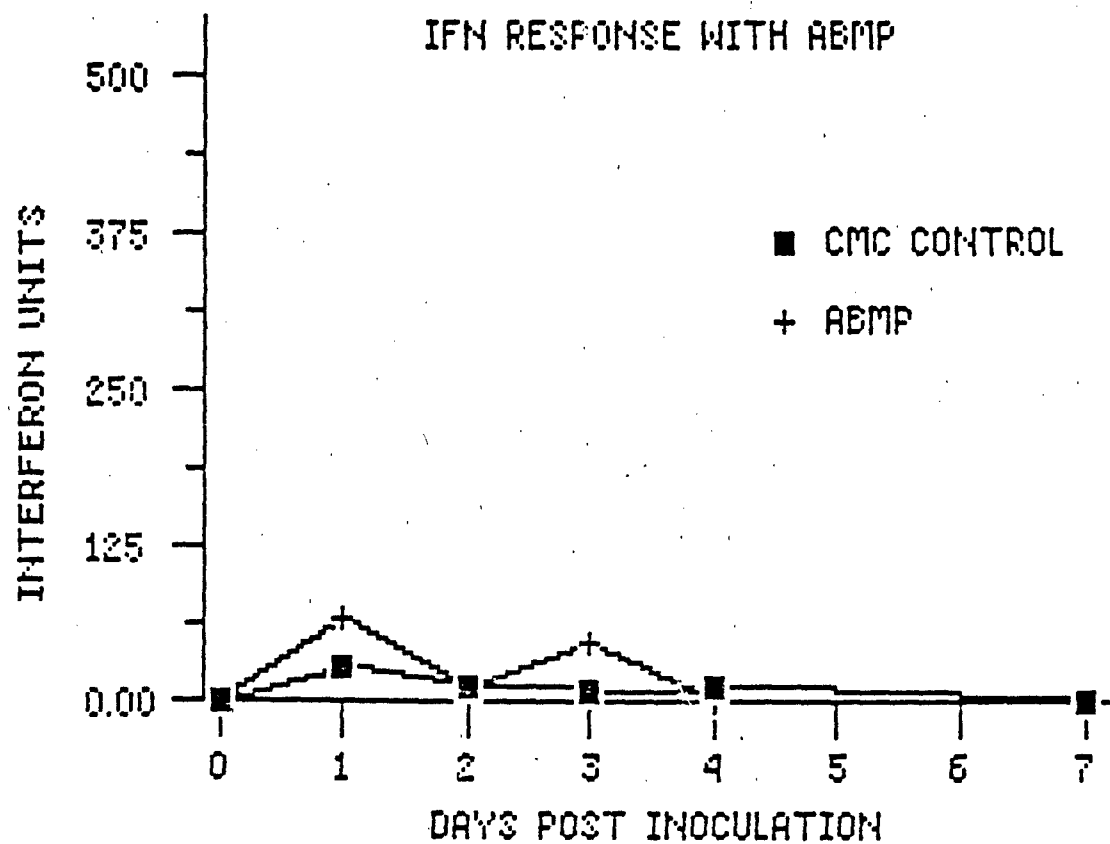


Figure 2. Serum interferon levels following treatment with ABMP. Mice were injected ip with 0.2ml CMC or 250 mg/kg drug in 0.2 ml CMC and then bled on days indicated. Day 0 bleed was obtained immediately before injection. Each point represents a mean of three mice.

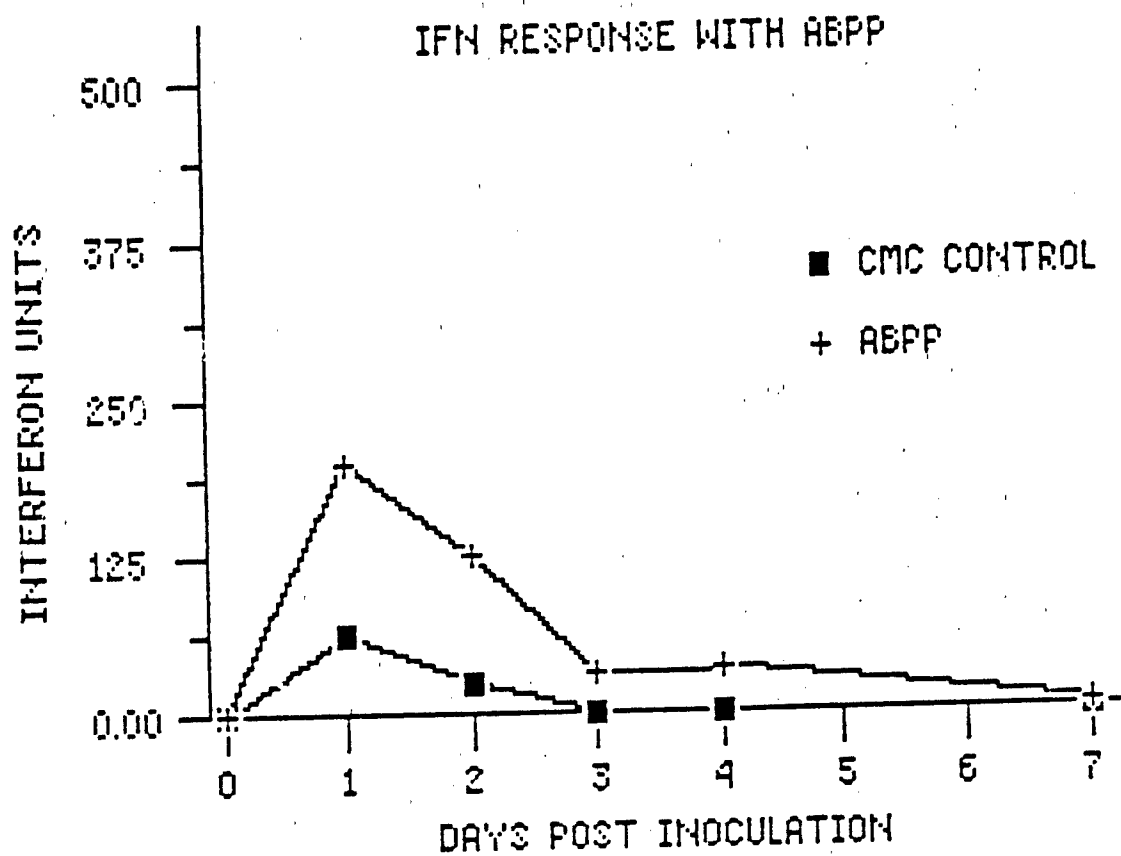


Figure 3. Serum interferon levels following treatment with ABPP. Mice were injected ip with 0.2ml CMC or 250 mg/kg drug in 0.2 ml CMC and then bled on days indicated. Day 0 bleed was obtained immediately before injection. Each point represents a mean of three mice.

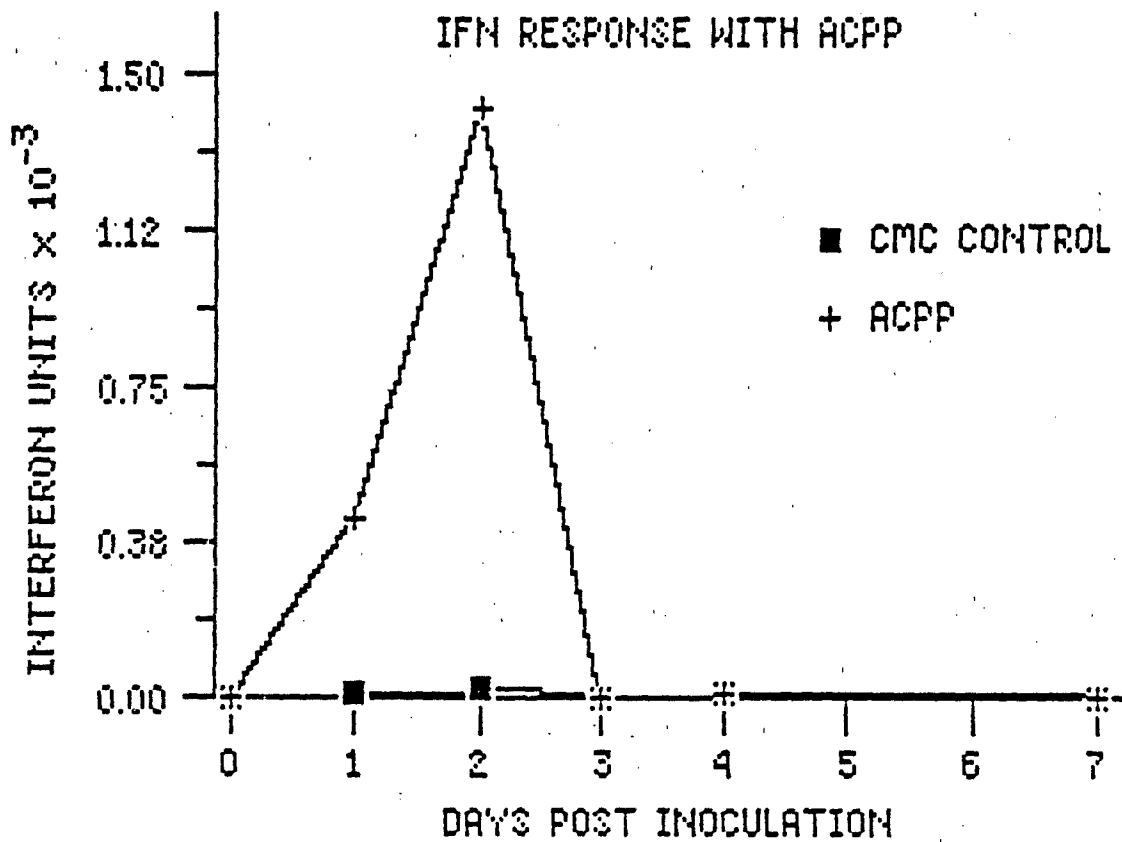


Figure 4. Serum interferon levels following treatment with ACP. Mice were injected ip with 0.2ml CMC or 250 mg/kg drug in 0.2 ml CMC and then bled on days indicated. Day 0 bleed was obtained immediately before injection. Each point represents a mean of three mice.

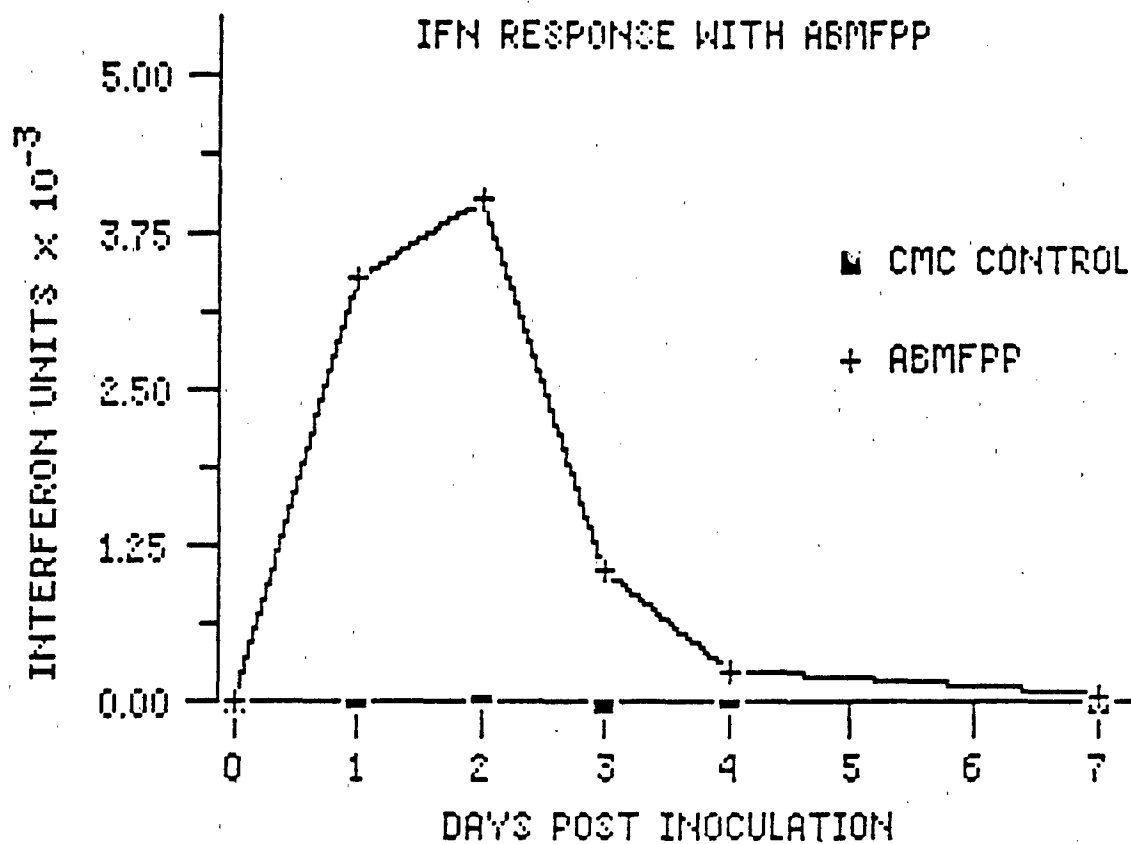
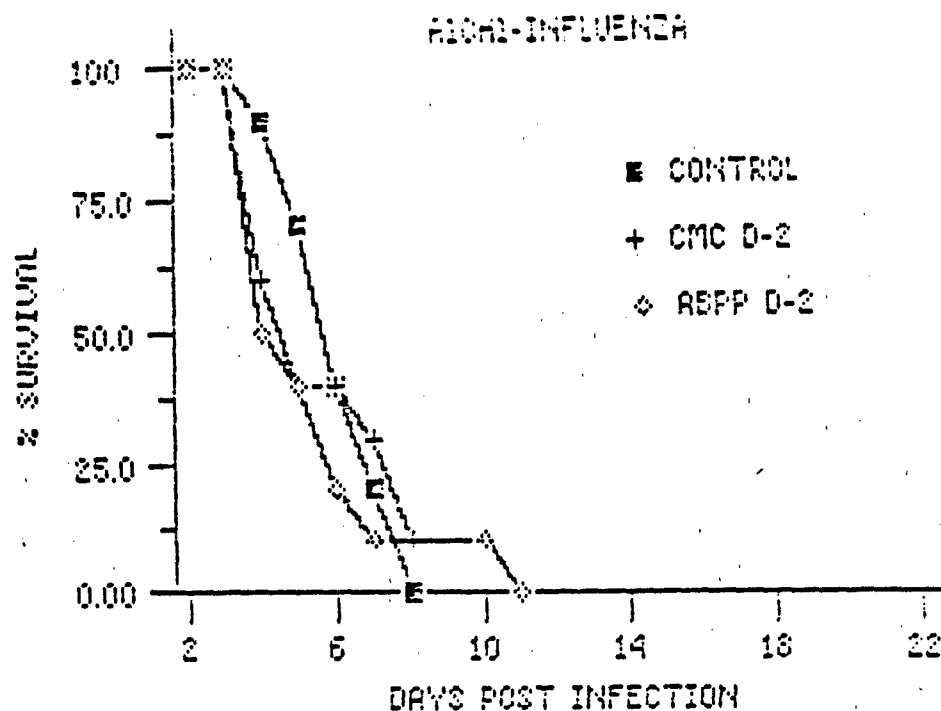


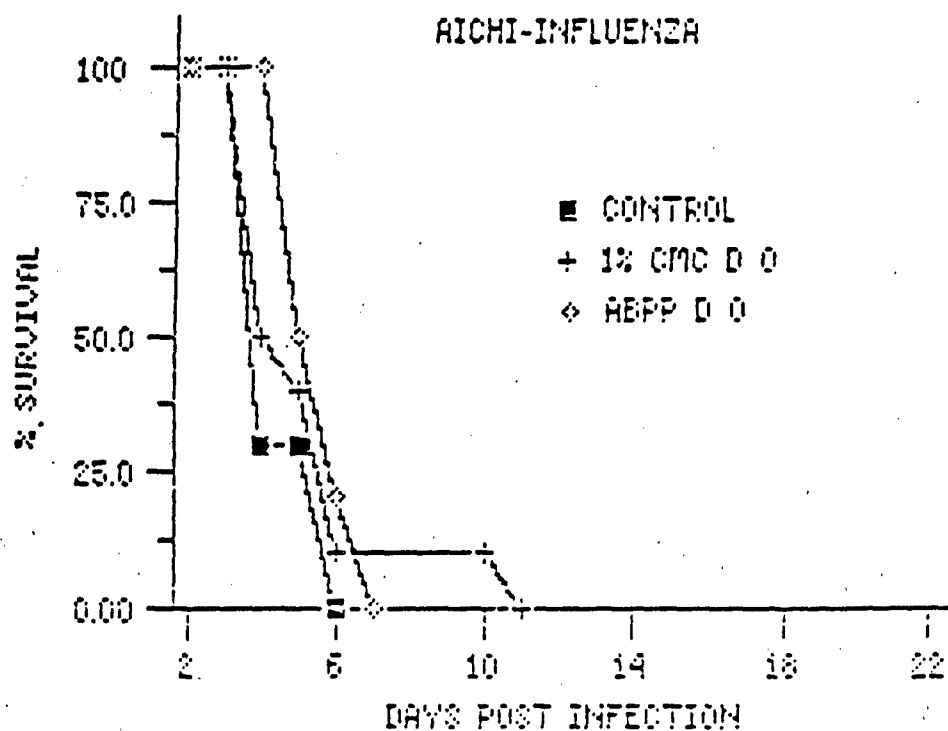
Figure 5. Serum interferon levels following treatment with ABMFPP. Mice were injected ip with 0.2ml CMC or 250 mg/kg drug in 0.2 ml CMC and then bled on days indicated. Day 0 bleed was obtained immediately before injection. Each point represents a mean of three mice.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.96	-
ABPP Day -2	5.21	NS
Saline Control	6.07	NS

Figure 6. Effect of ABPP, given on day -2, on resistance to influenza-induced pneumonitis.

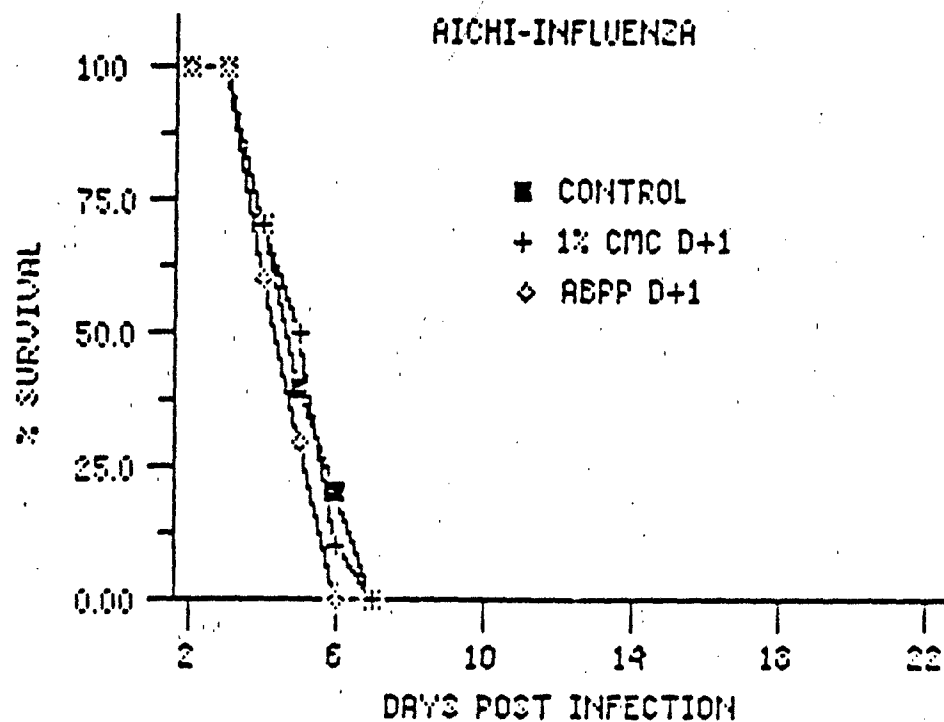
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.11	-
ABPP Day 0	5.65	NS
Saline Control	4.52	NS

Figure 7. Effect of ABPP, given on day 0, on resistance to influenza-induced pneumonitis.

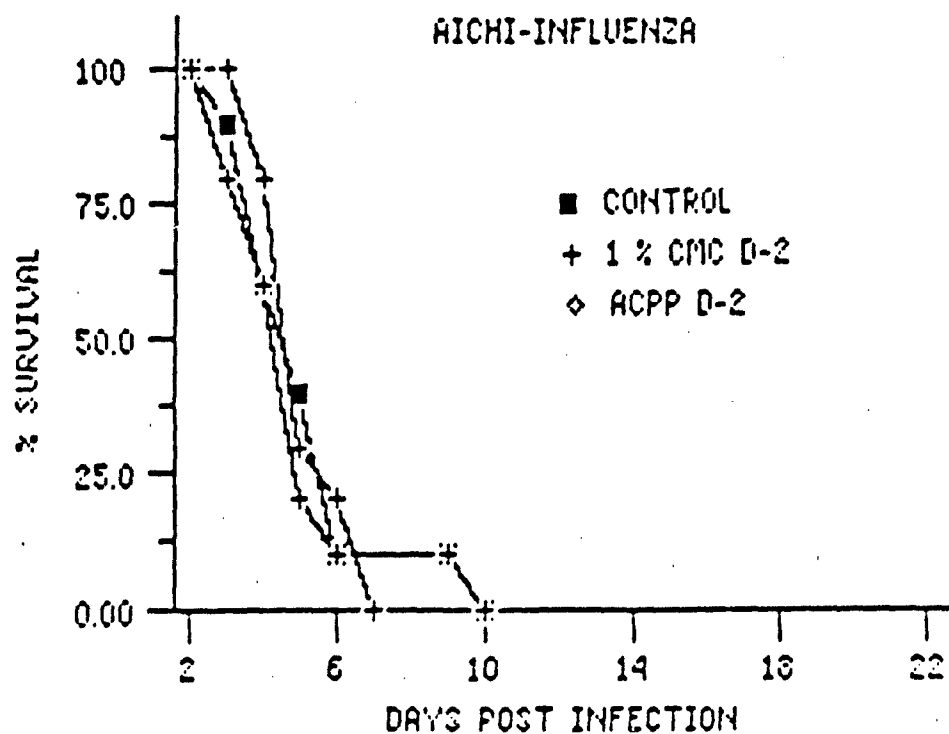
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.20	-
ABPP Day +1	4.83	NS
Saline Control	5.19	NS

Figure 8. Effect of ABPP, given on day +1, on resistance to influenza-induced pneumonitis.

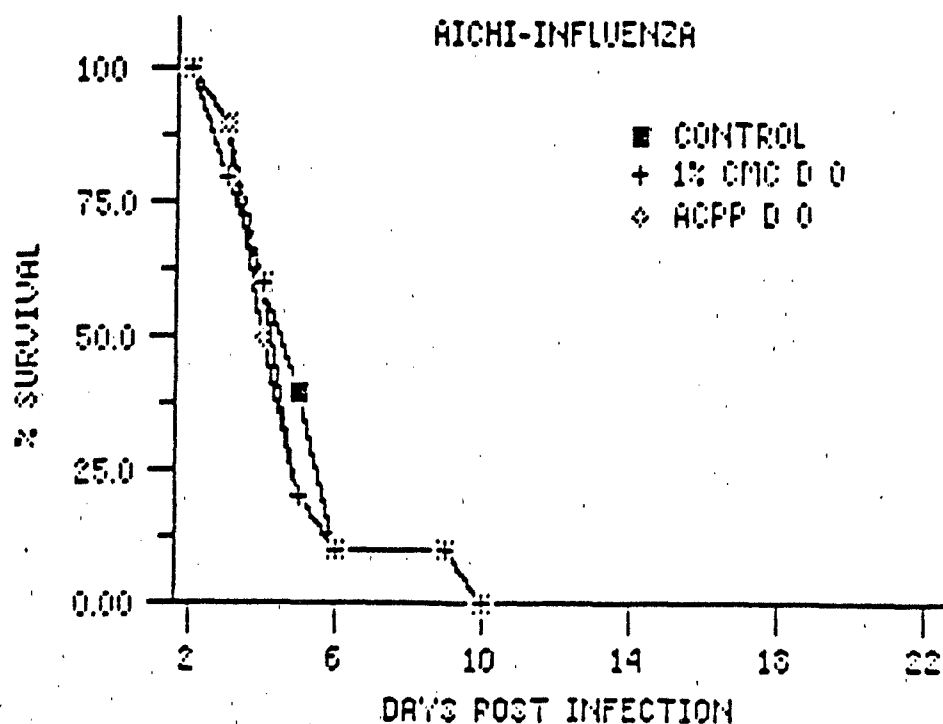
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.21	-
ACPD Day -2	5.21	NS
Saline Control	4.80	NS

Figure 9. Effect of ACPD, given on day -2, on resistance to influenza-induced pneumonitis.

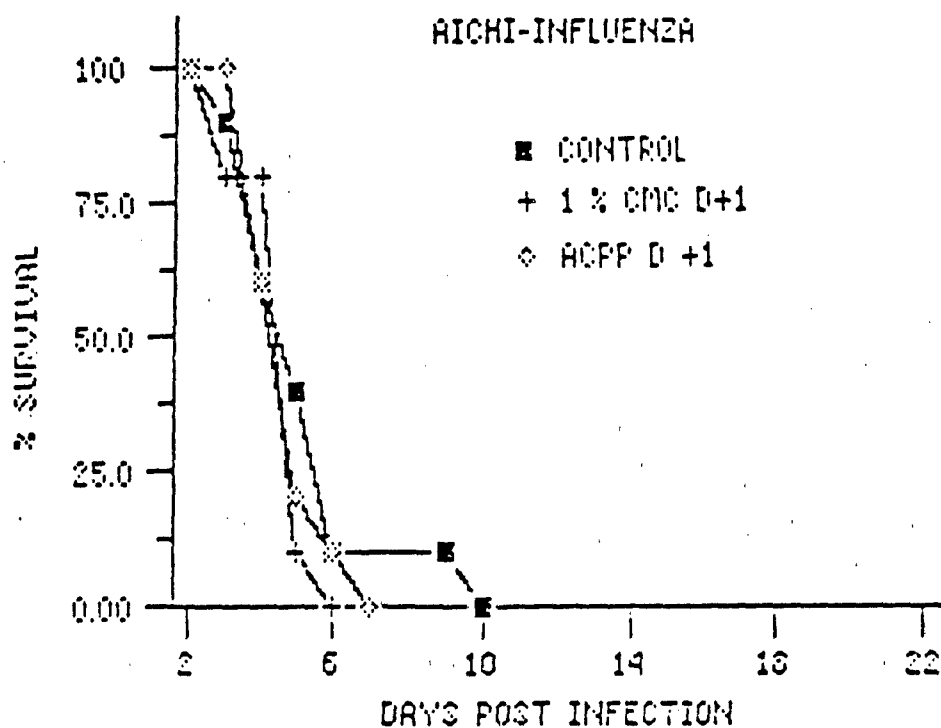
Mice were given ACPD (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two day prior to (D -2) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	4.61	--
ACPP Day 0	4.74	NS
Saline Control	4.80	NS

Figure 10. Effect of ACPD, given on day 0, on resistance to influenza-induced pneumonitis.

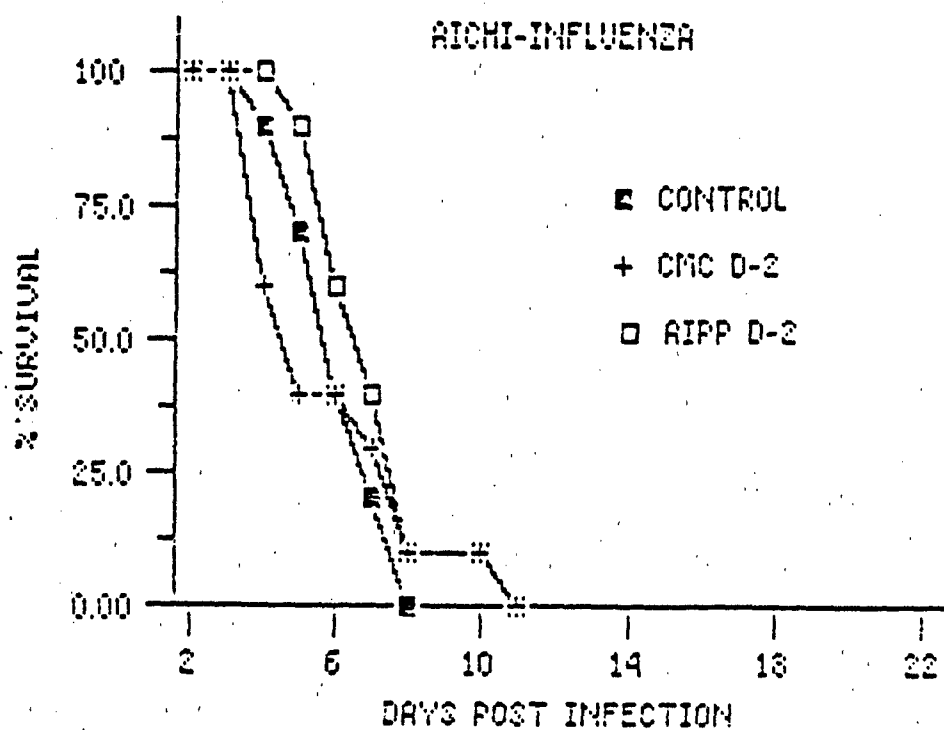
Mice were given ACPD (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	4.60	-
ACPD Day +1	4.82	NS
Saline Control	4.80	NS

Figure 11. Effect of ACPD, given on day +1, on resistance to influenza-induced pneumonitis.

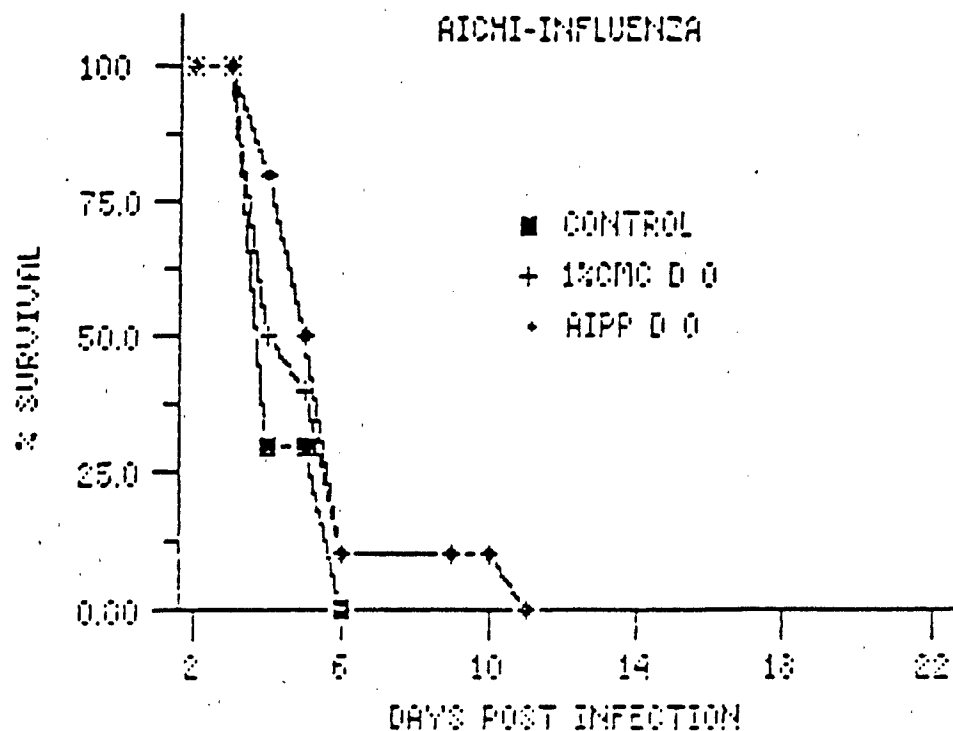
Mice were given ACPD (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.96	-
AIPP Day -2	7.04	NS
Saline Control	6.07	NS

Figure 12. Effect of AIPP, given on day -2, on resistance to influenza-induced pneumonitis.

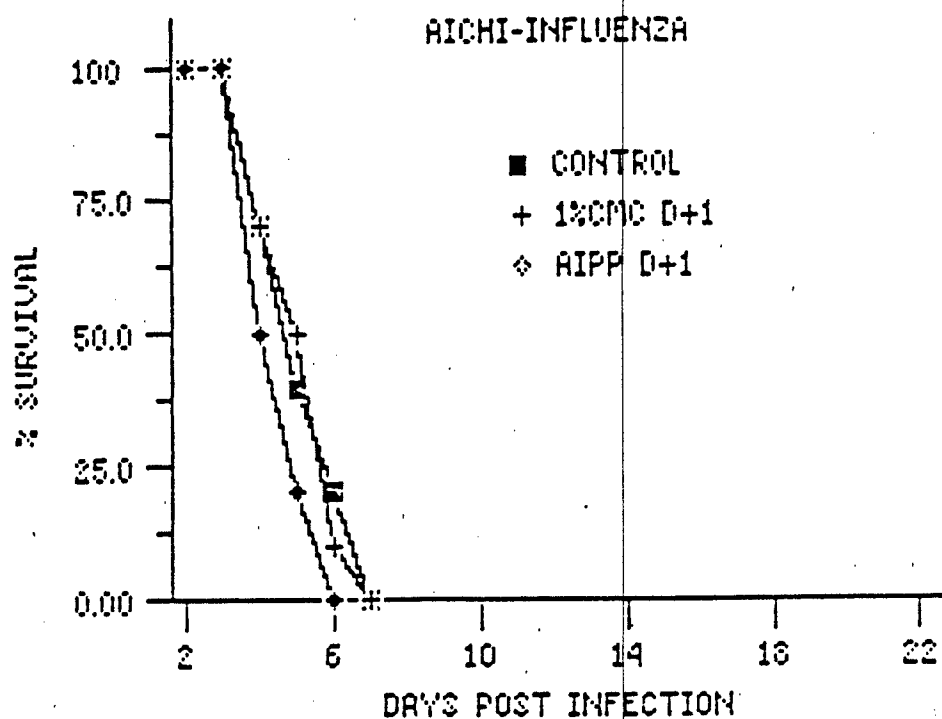
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.11	-
AIPP Day 0	5.57	NS
Saline Control	4.52	NS

Figure 13. Effect of AIPP, given on day 0, on resistance to influenza-induced pneumonitis.

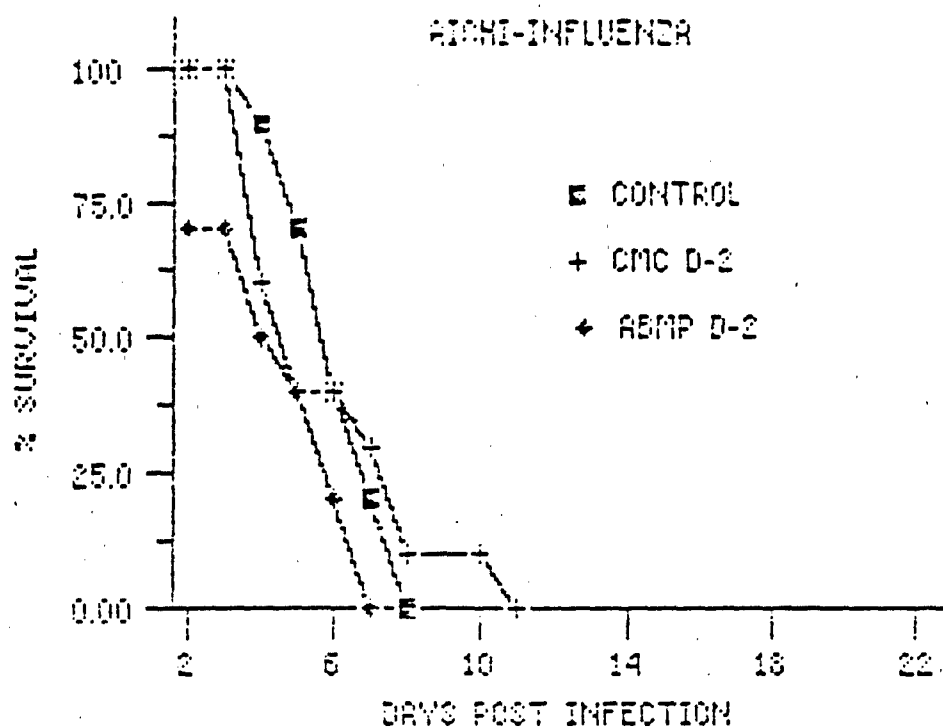
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.20	-
AIPP Day +1	4.87	NS
Saline Control	5.19	NS

Figure 14. Effect of AIPP, given on day +1, on resistance to influenza-induced pneumonitis.

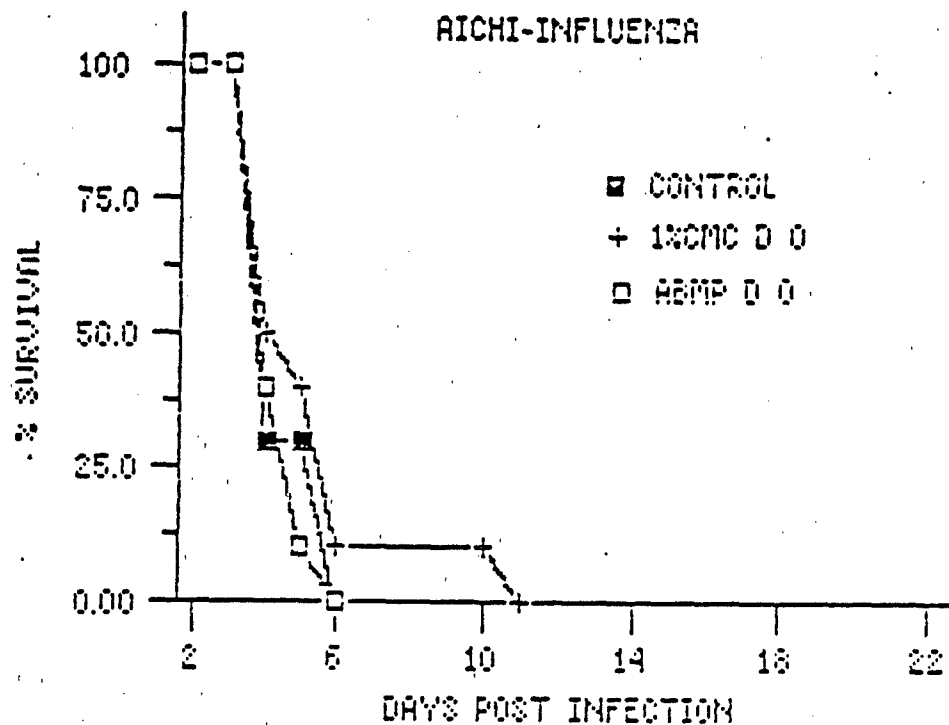
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.96	-
ABMP Day -2	5.38	NS
Saline Control	6.07	NS

Figure 15. Effect of ABMP, given on day -2, on resistance to influenza-induced pneumonitis.

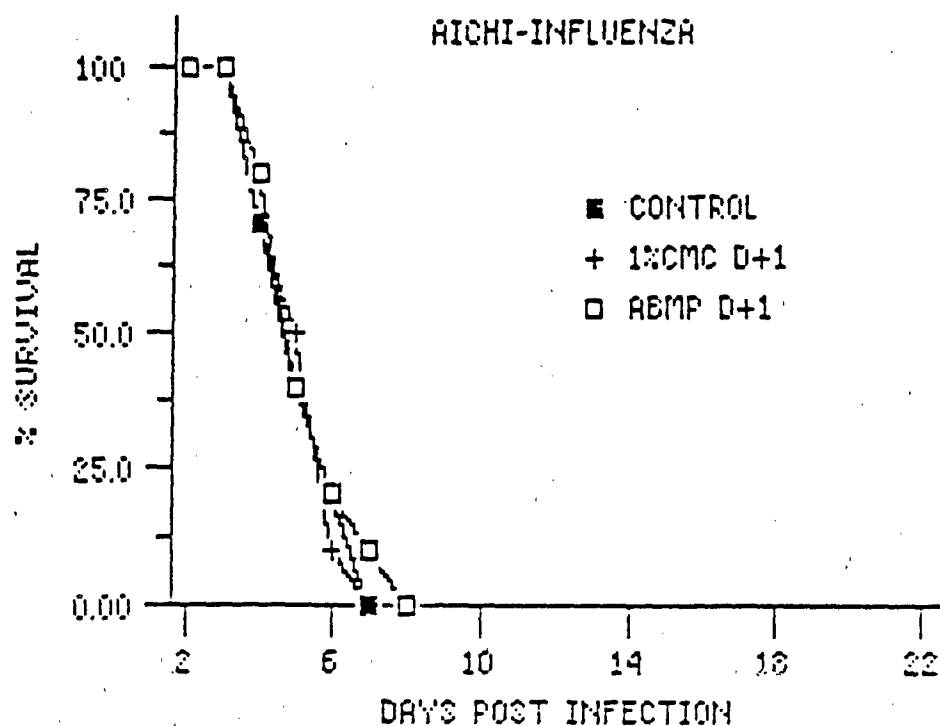
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.11	-
ABMP Day 0	4.45	NS
Saline Control	4.52	NS

Figure 16. Effect of ABMP, given on day 0, on resistance to influenza-induced pneumonitis.

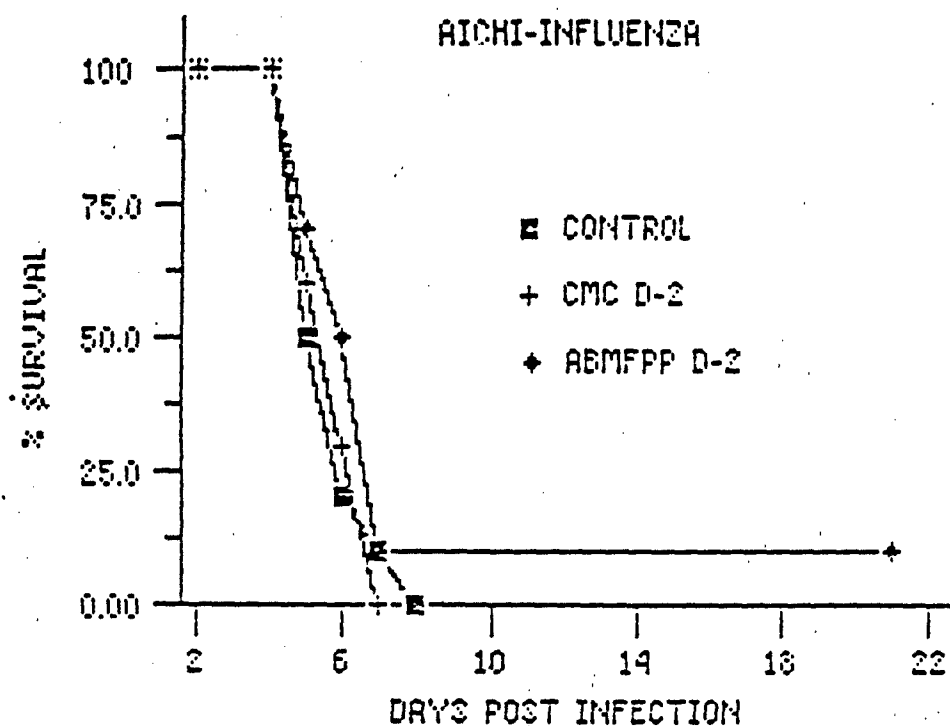
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip. on the day of (D 0) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.20	-
ABMP Day +1	5.38	NS
Saline Control	5.19	NS

Figure 17. Effect of ABMP, given on day +1, on resistance to influenza-induced pneumonitis.

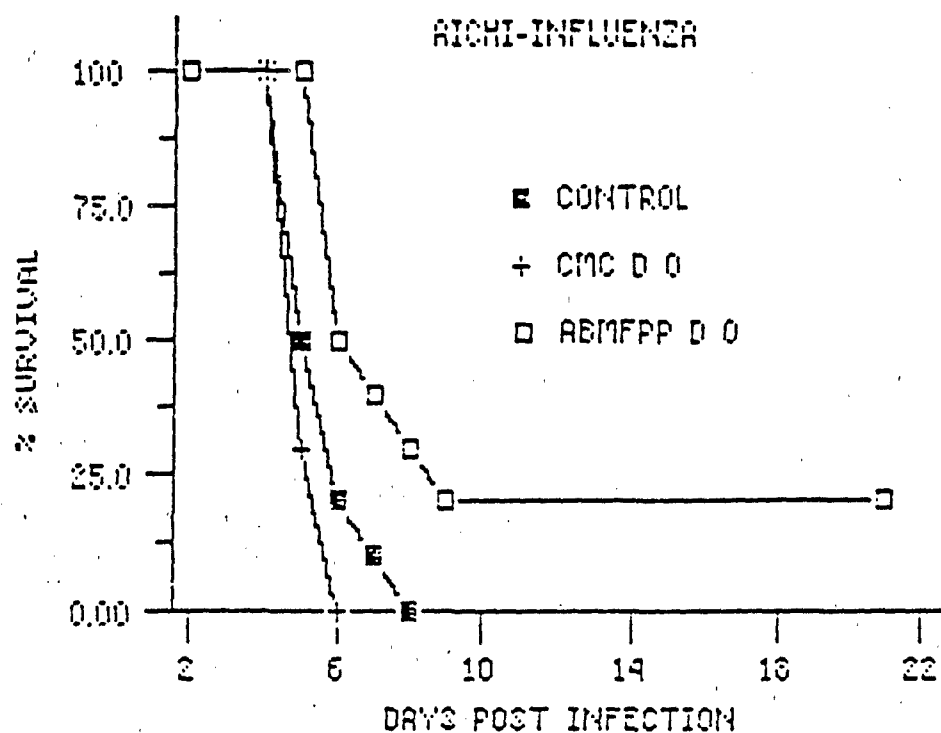
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.84	-
ABMFPP Day -2	6.85	NS
Saline Control	5.72	NS

Figure 18. Effect of ABMFPP, given on day -2, on resistance to influenza-induced pneumonitis.

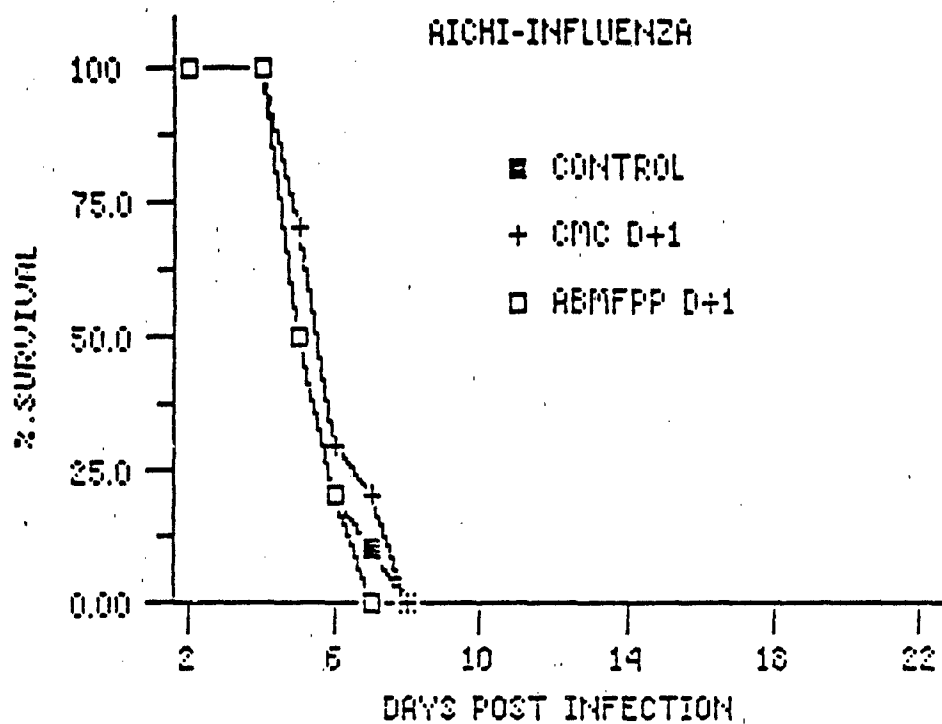
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.28	-
ABMFPP Day 0	8.39	<0.02
Saline Control	5.72	NS

Figure 19. Effect of ABMFPP, given on day 0, on resistance to influenza-induced pneumonitis.

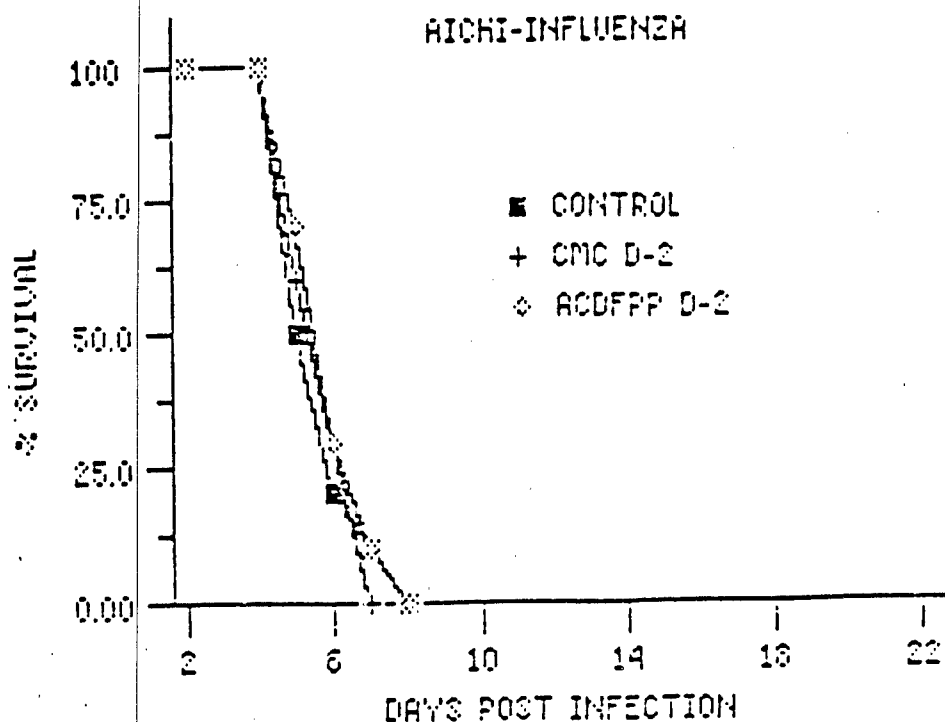
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.0	-
ABMFPP Day +1	5.65	NS
Saline Control	5.72	NS

Figure 20. Effect of ABMFPP, given on day +1, on resistance to influenza-induced pneumonitis.

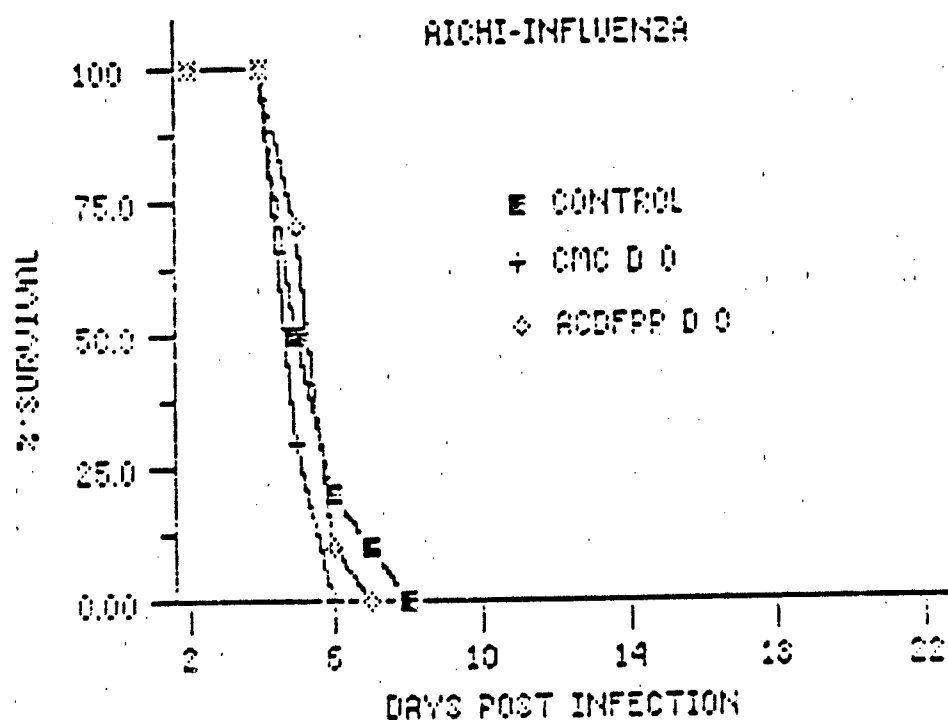
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.84	-
ACDFPP Day -2	6.03	NS
Saline Control	5.72	NS

Figure 21. Effect of ACDFPP, given on day -2, on resistance to influenza-induced pneumonitis.

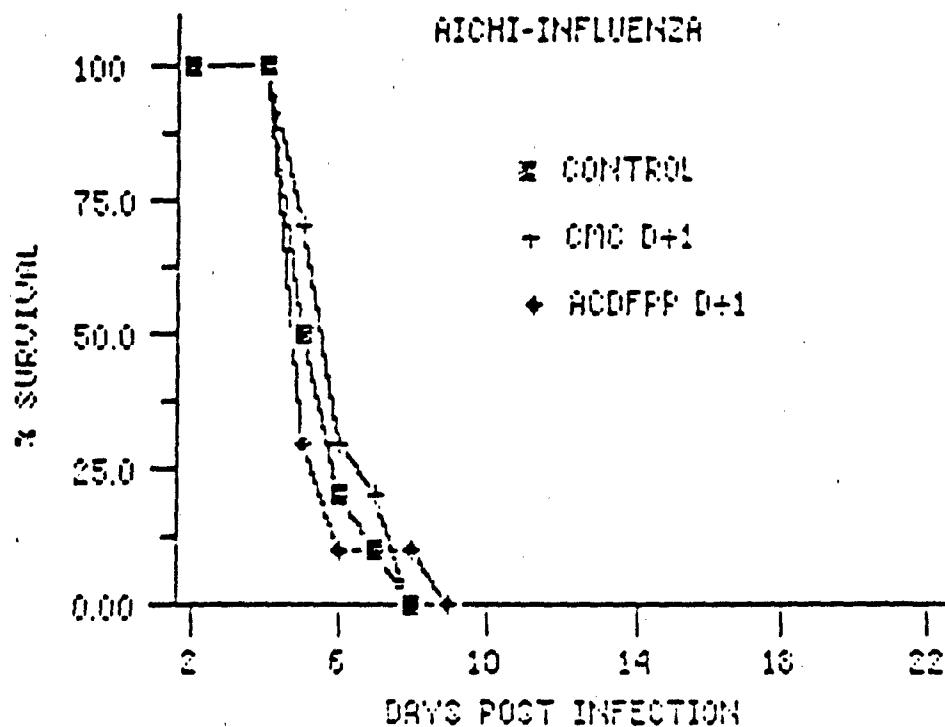
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.28	-
ACDFFP Day 0	5.77	NS
Saline Control	5.72	NS

Figure 22. Effect of ACDFFP, given on day 0, on resistance to influenza-induced pneumonitis.

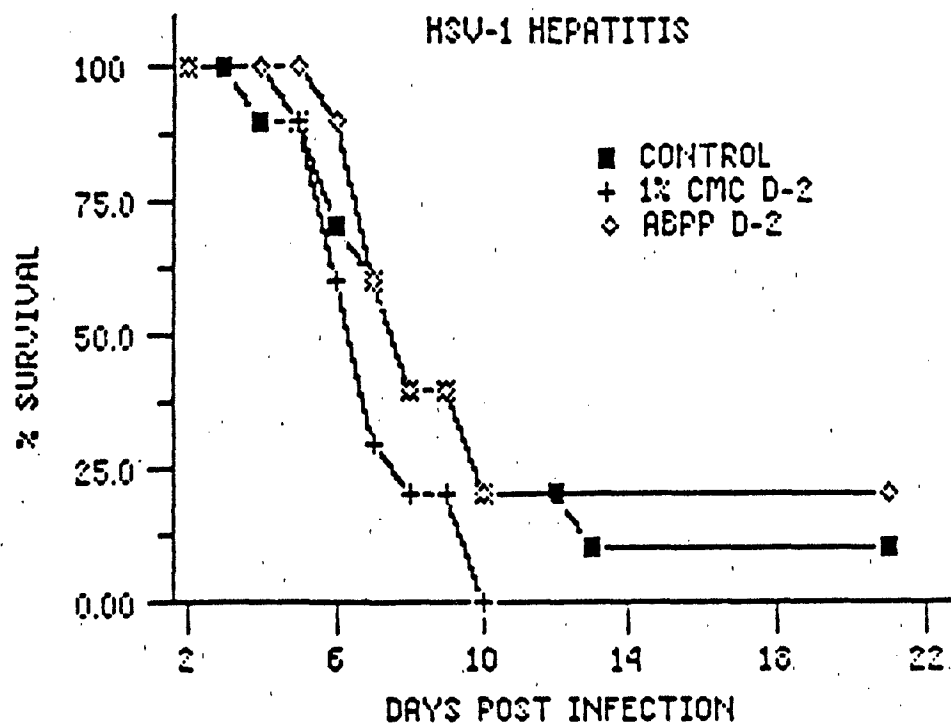
Mice were given ACDFFP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.11	-
ACDFFP Day +1	5.50	NS
Saline Control	5.72	NS

Figure 23. Effect of ACDFFP, given on day +1, on resistance to influenza-induced pneumonitis.

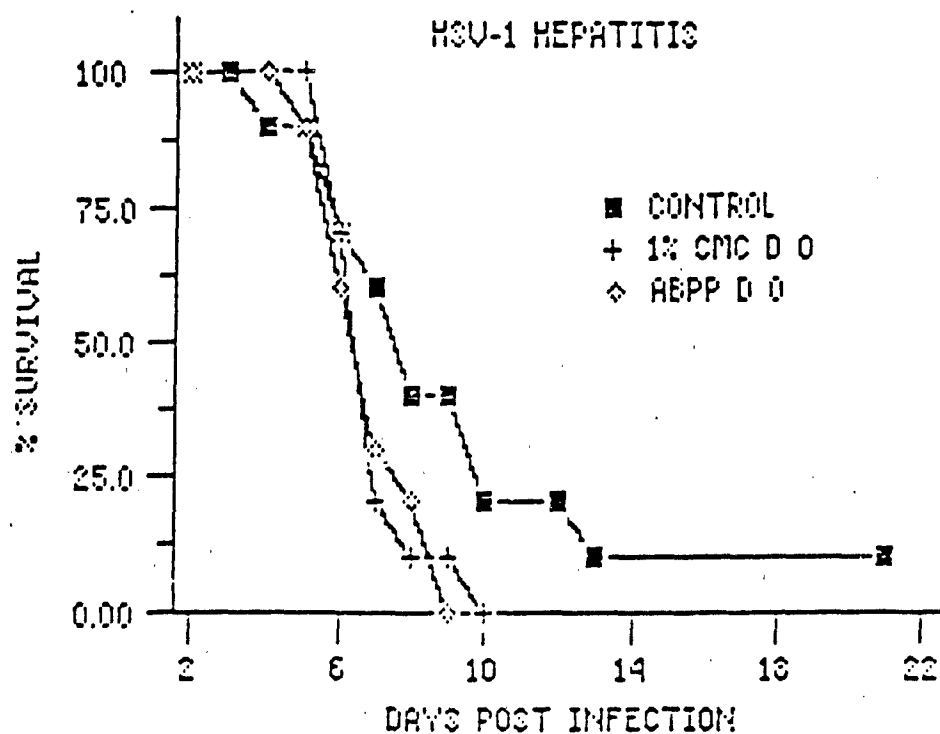
Mice were given ACDFFP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one after (D +1) intranasal challenge with 10 LD₅₀ of influenza virus (Aichi Strain). Control animals received 1% CMC on D +1. A saline control group was also include NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.03	-
ABPP Day -2	9.47	NS
Saline Control	8.60	NS

Figure 24. Effect of ABPP, given on day -2, on resistance to herpesvirus-induced hepatitis.

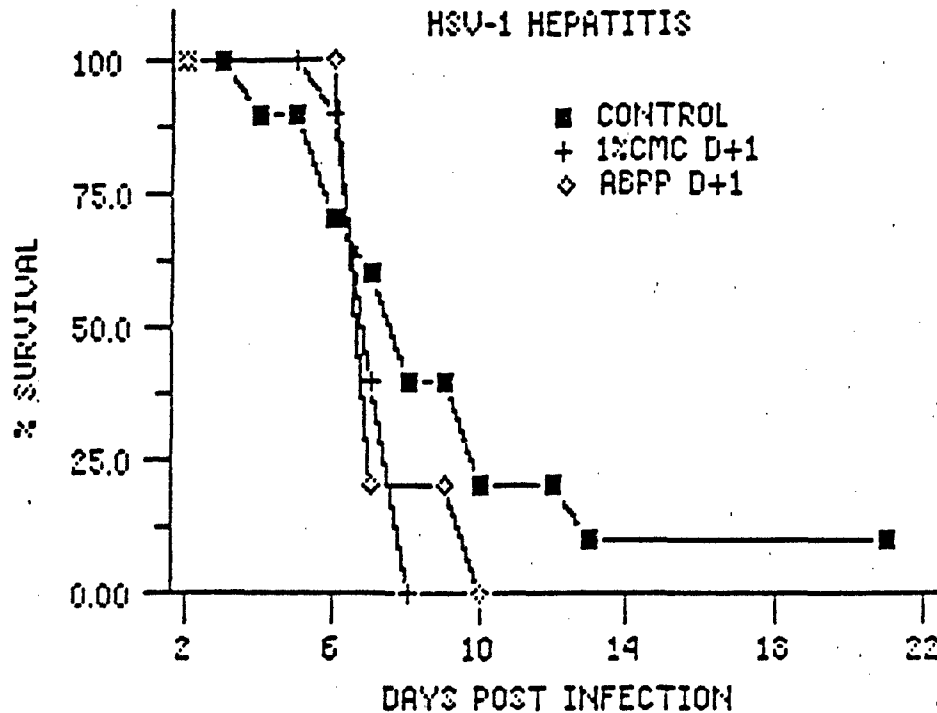
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.02	-
ABPP Day 0	6.89	<0.02
Saline Control	8.60	NS

Figure 25. Effect of ABPP, given on day 0, on resistance to herpesvirus-induced hepatitis.

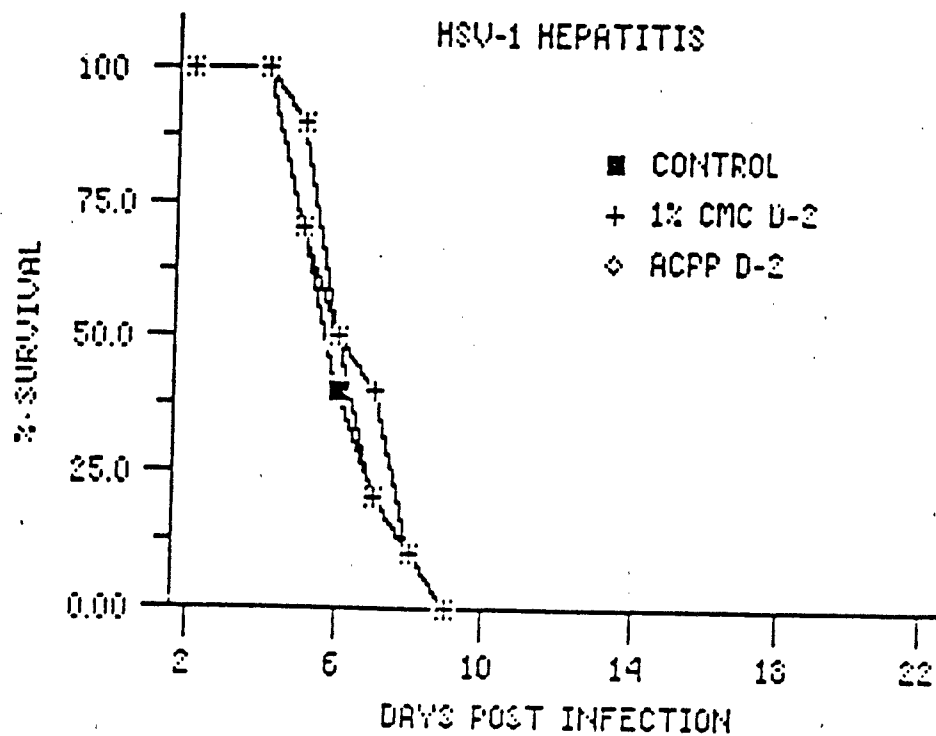
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.27	-
ABPP Day +1	7.52	NS
Saline Control	8.60	NS

Figure 26. Effect of ABPP, given on day +1, on resistance to herpesvirus-induced hepatitis.

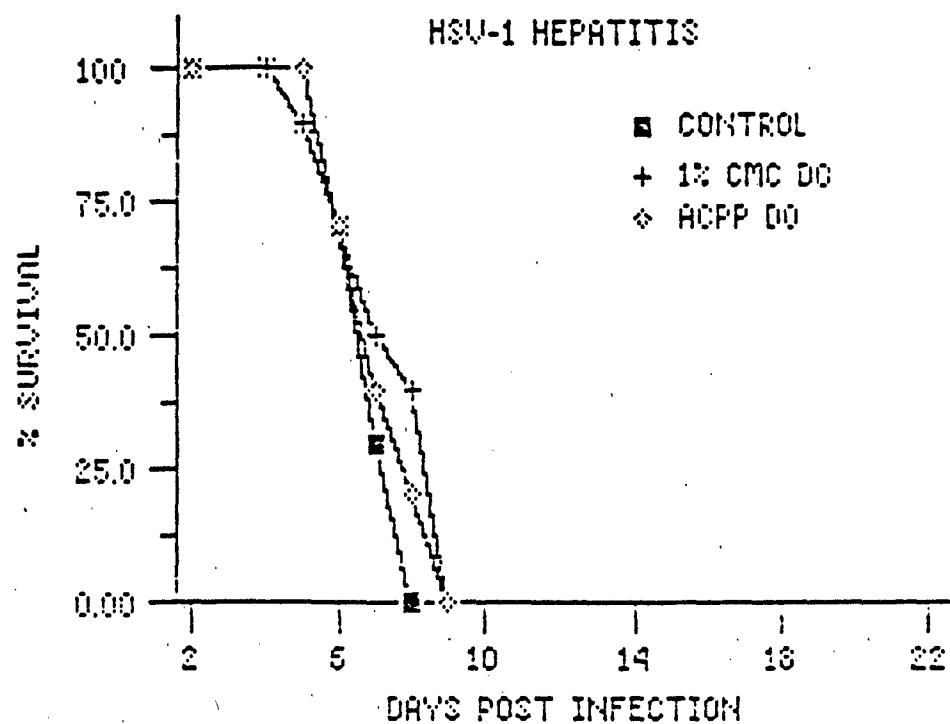
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.61	-
ACPD Day -2	6.55	NS
Saline Control	6.28	NS

Figure 27. Effect of ACPD, given on day -2, on resistance to herpesvirus-induced hepatitis.

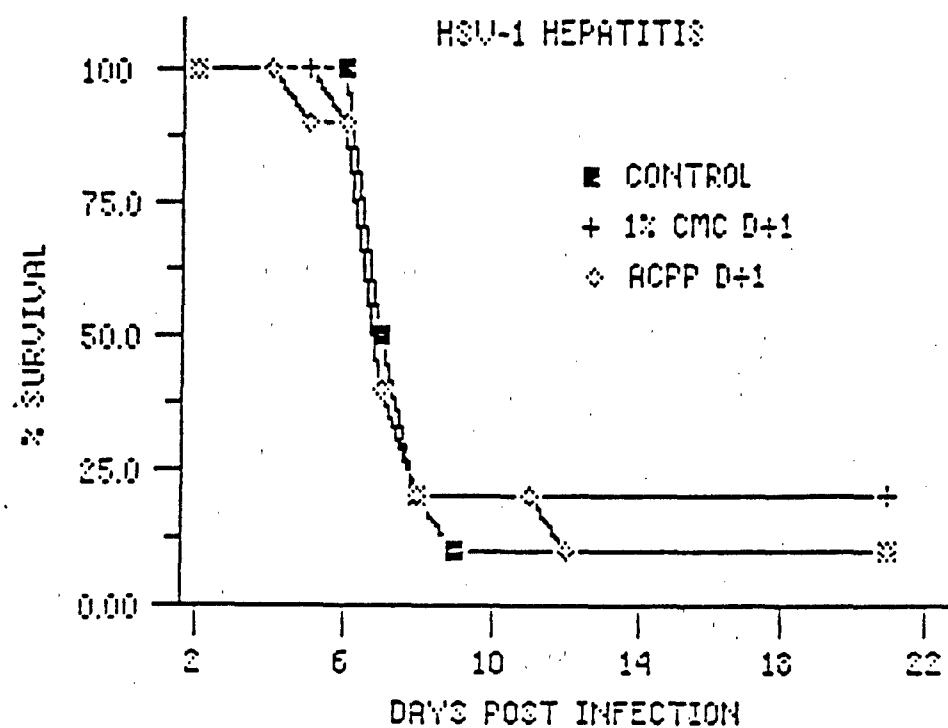
Mice were given ACPD (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.35	-
ACPD Day 0	7.22	NS
Saline Control	6.83	NS

Figure 28. Effect of ACPD, given on day 0, on resistance to herpesvirus-induced hepatitis.

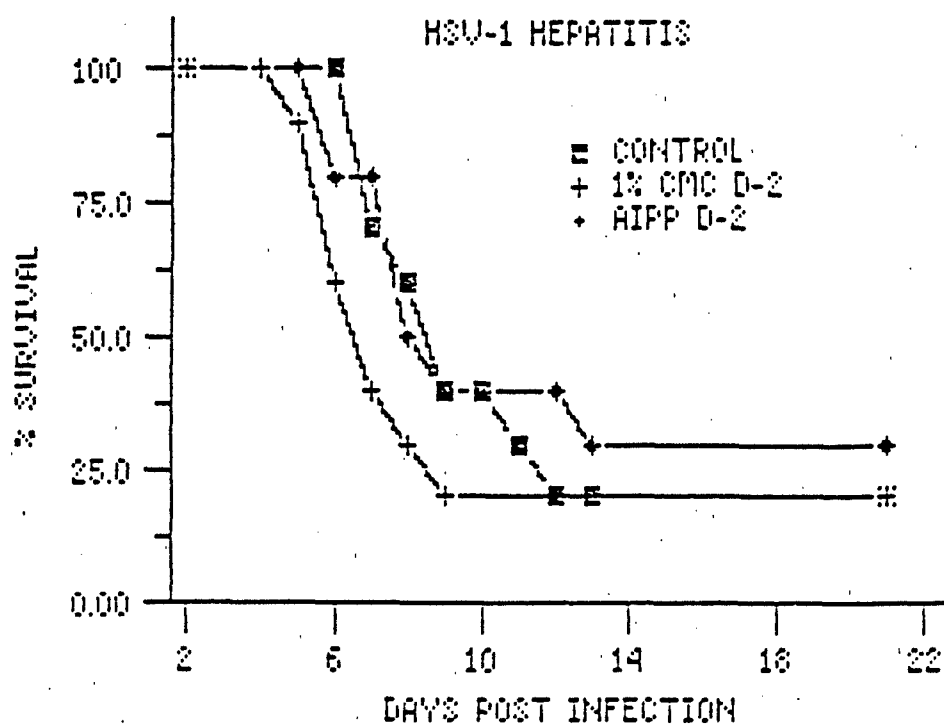
Mice were given ACPD (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.82	-
ACPD Day +1	8.19	NS
Saline Control	8.34	NS

Figure 29. Effect of ACPD, given on day +1, on resistance to herpesvirus-induced hepatitis.

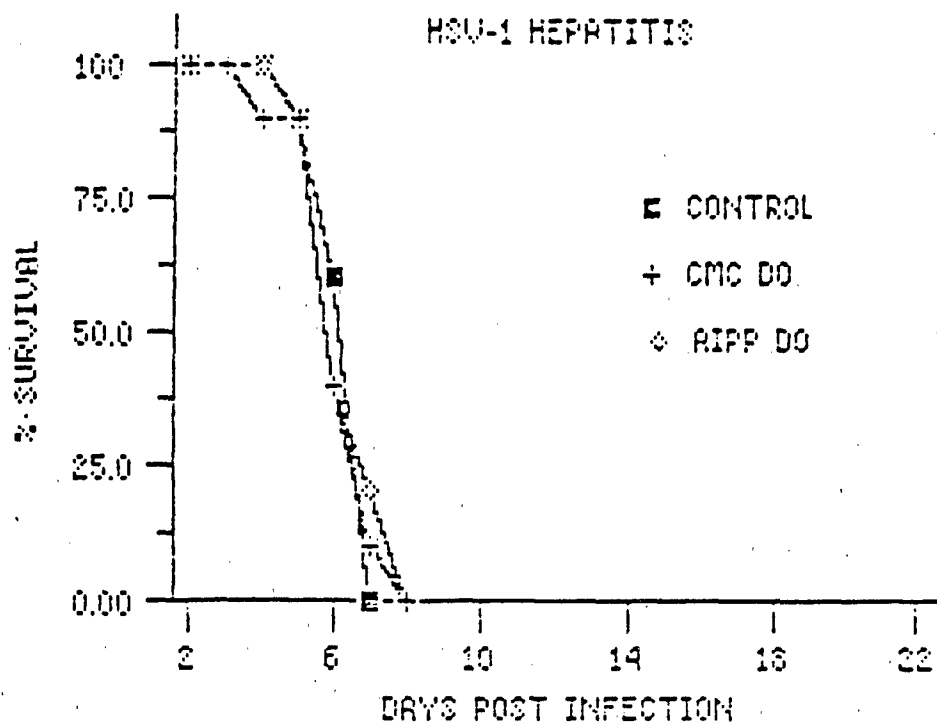
Mice were given ACPD (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.37	-
AIPP Day -2	10.72	NS
Saline Control	10.26	NS

Figure 30. Effect of AIPP, given on day -2, on resistance to herpesvirus-induced hepatitis.

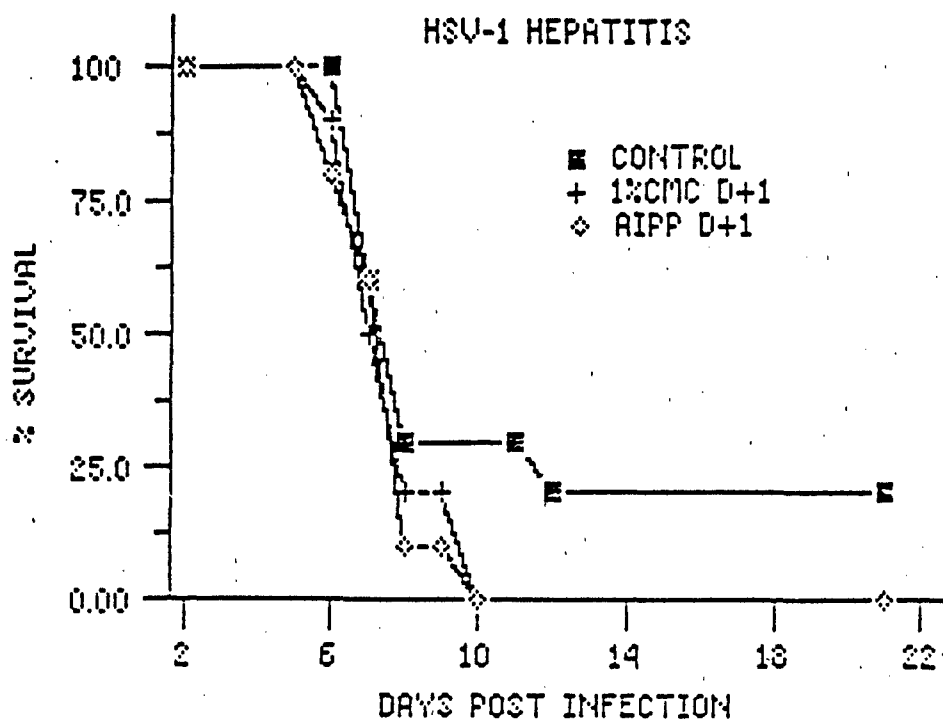
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.21	-
AIPP Day 0	6.44	NS
Saline Control	6.46	NS

Figure 31. Effect of AIPP, given on day 0, on resistance to herpesvirus-induced hepatitis.

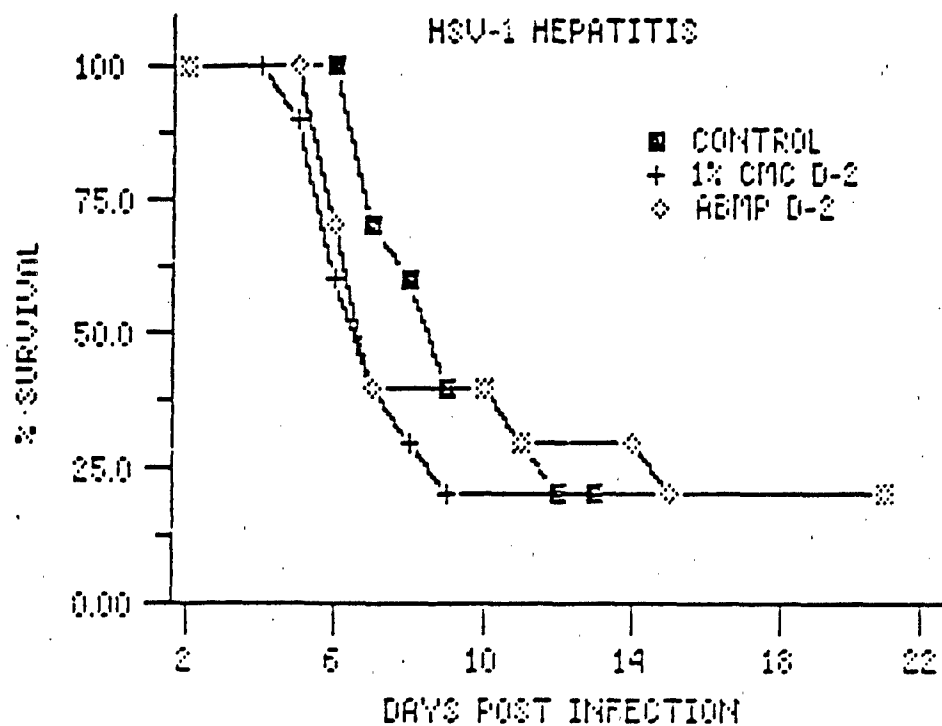
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.85	-
AIPP Day +1	7.52	NS
Saline Control	9.69	NS

Figure 32. Effect of AIPP, given on day +1, on resistance to herpesvirus-induced hepatitis.

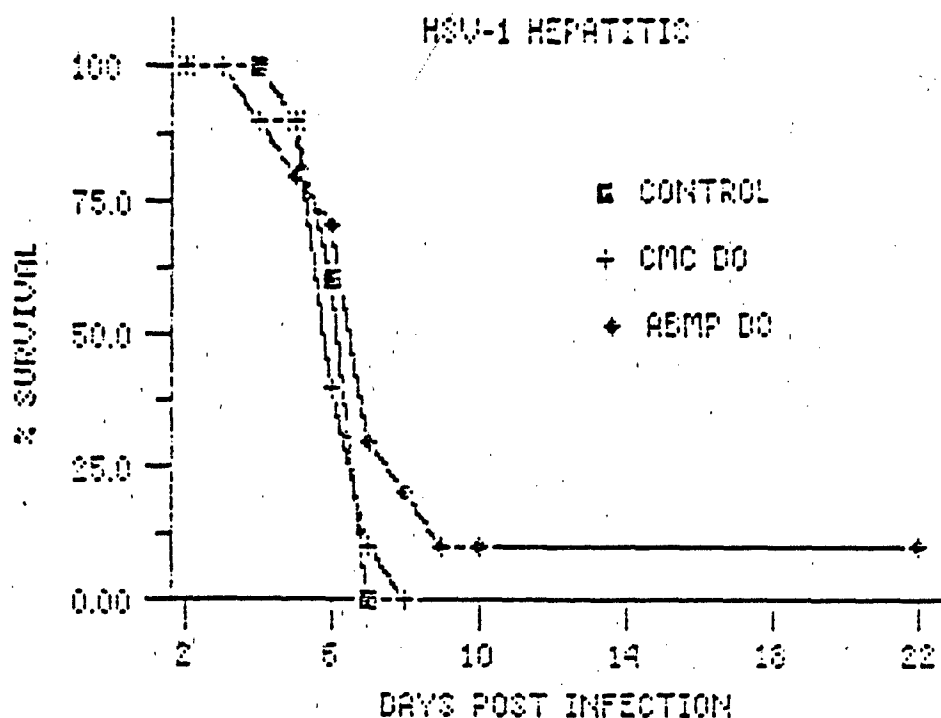
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.37	-
ABMP Day -2	9.40	NS
Saline Control	10.26	NS

Figure 33. Effect of ABMP, given on day -2, on resistance to herpesvirus-induced hepatitis.

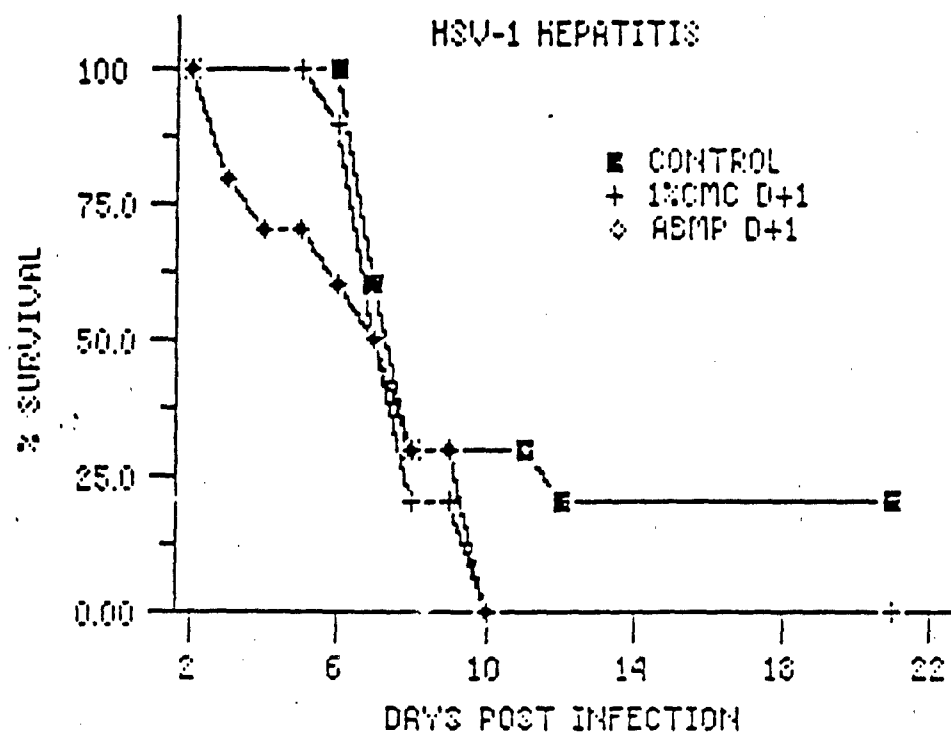
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.21	-
ABMP Day 0	7.31	NS
Saline Control	6.46	NS

Figure 34. Effect of ABMP, given on day 0, on resistance to herpesvirus-induced hepatitis.

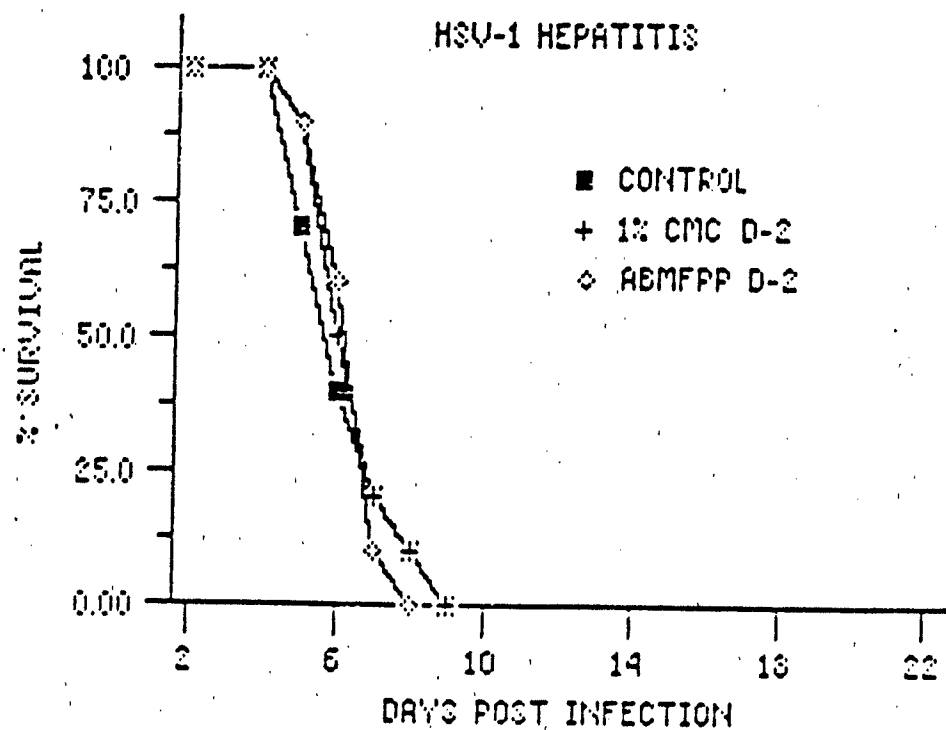
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.85	-
ABMP Day +1	5.63	NS
Saline Control	9.69	NS

Figure 35. Effect of ABMP, given on day +1, on resistance to herpesvirus-induced hepatitis.

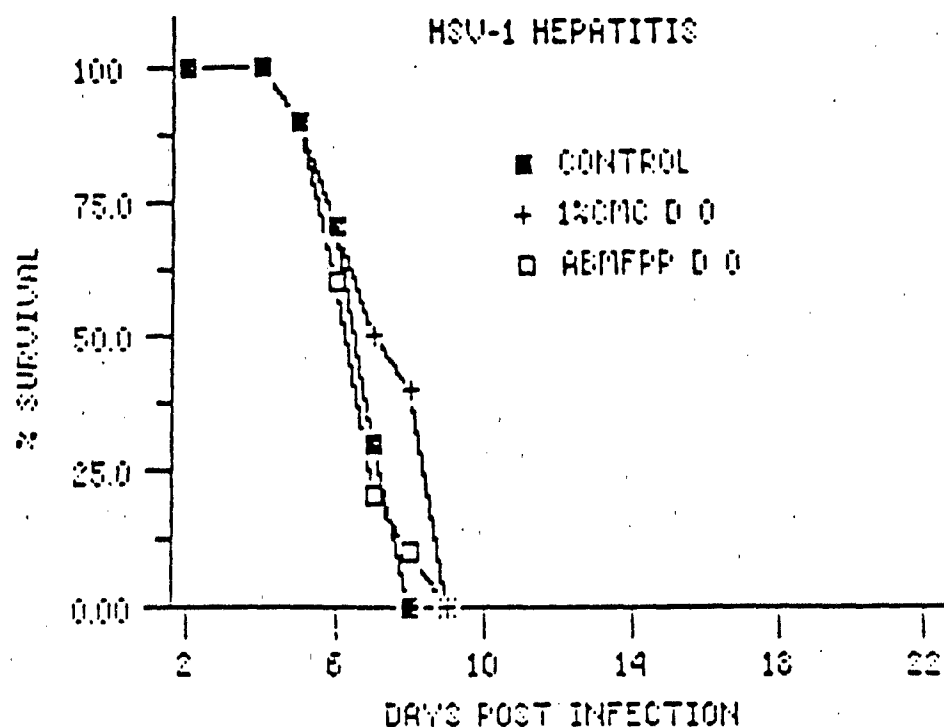
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.61	-
ABMFPP Day -2	6	NS
Saline Control	6.28	NS

Figure 36. Effect of ABMFPP, given on day -2, on resistance to herpesvirus-induced hepatitis.

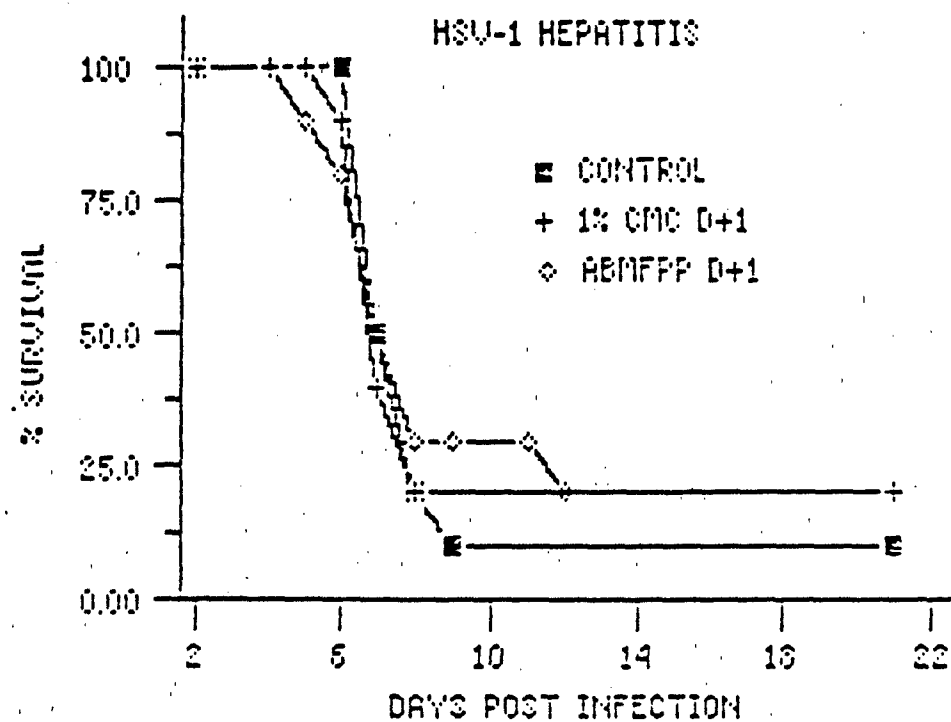
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.35	-
ABMFPP Day 0	6.72	NS
Saline Control	6.83	NS

Figure 37. Effect of ABMFPP, given on day 0, on resistance to herpesvirus-induced hepatitis.

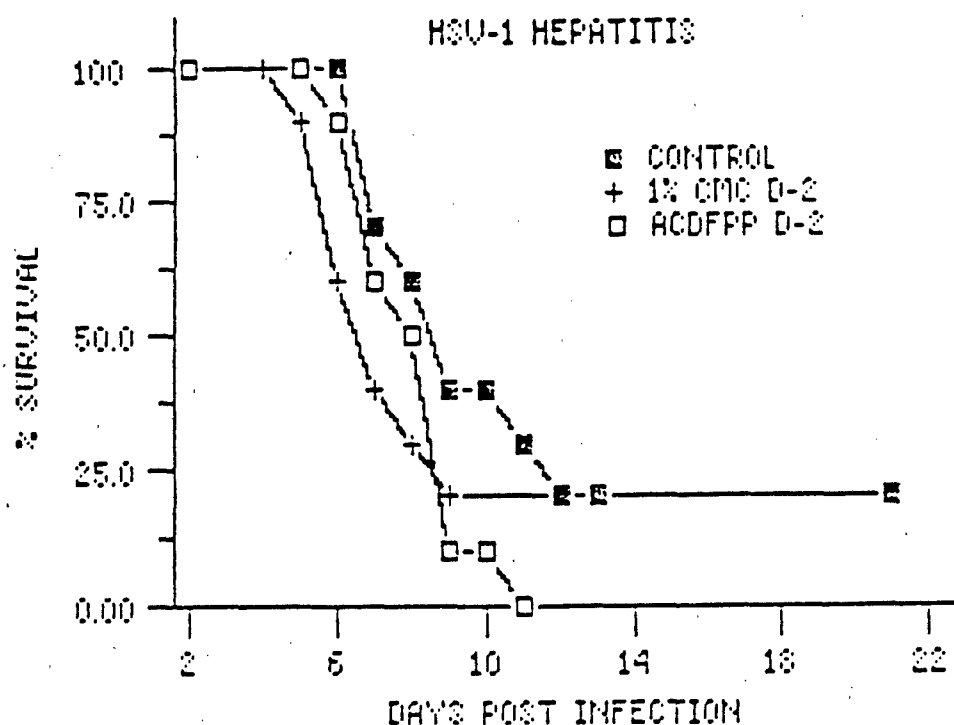
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = No Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.82	-
ABMFPP Day +1	9.17	NS
Saline Control	8.34	NS

Figure 38. Effect of ABMFPP, given on day +1, on resistance to herpesvirus-induced hepatitis.

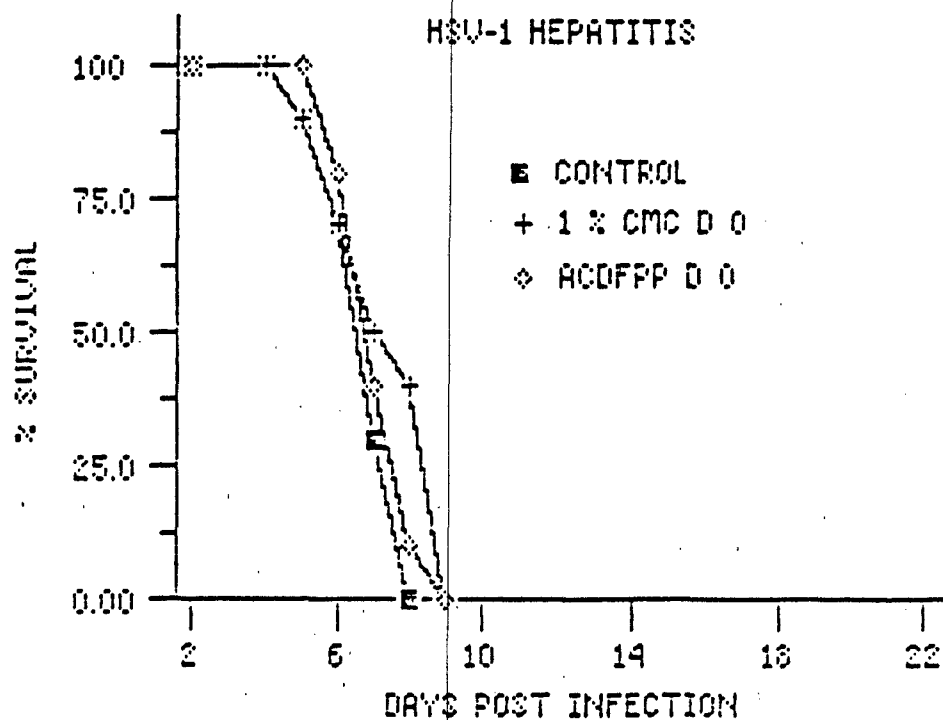
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.37	-
ACDFFP Day -2	8.08	NS
Saline Control	10.26	NS

Figure 39. Effect of ACDFFP, given on day -2, on resistance to herpesvirus-induced hepatitis.

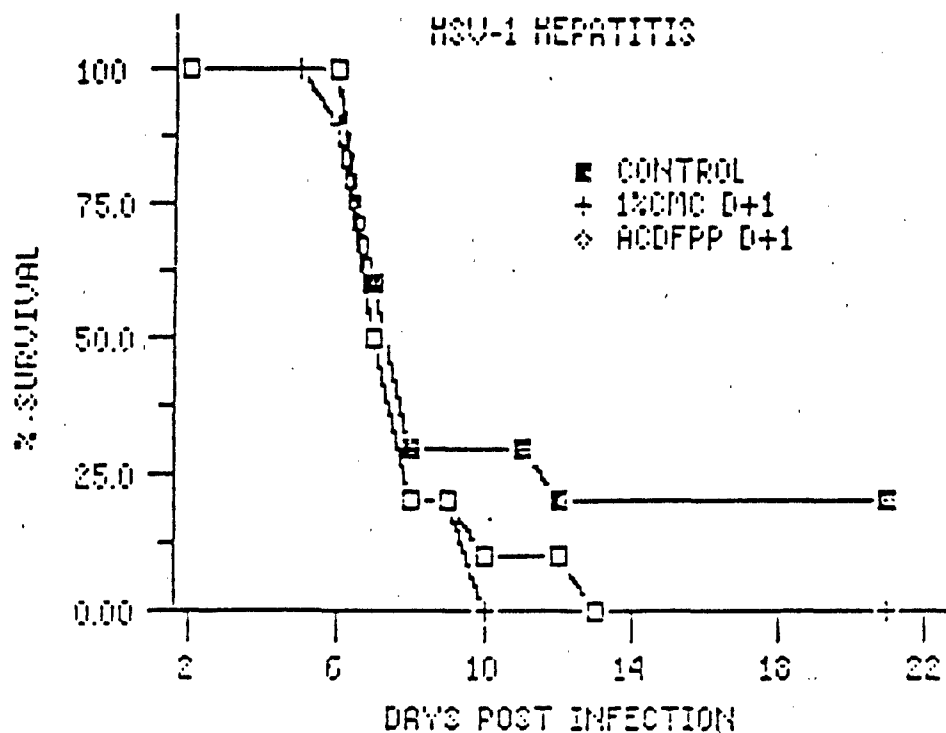
Mice were given ACDFFP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.35	-
ACDFPP Day 0	7.24	NS
Saline Control	6.83	NS

Figure 40. Effect of ACDFPP, given on day 0, on resistance to herpesvirus-induced hepatitis.

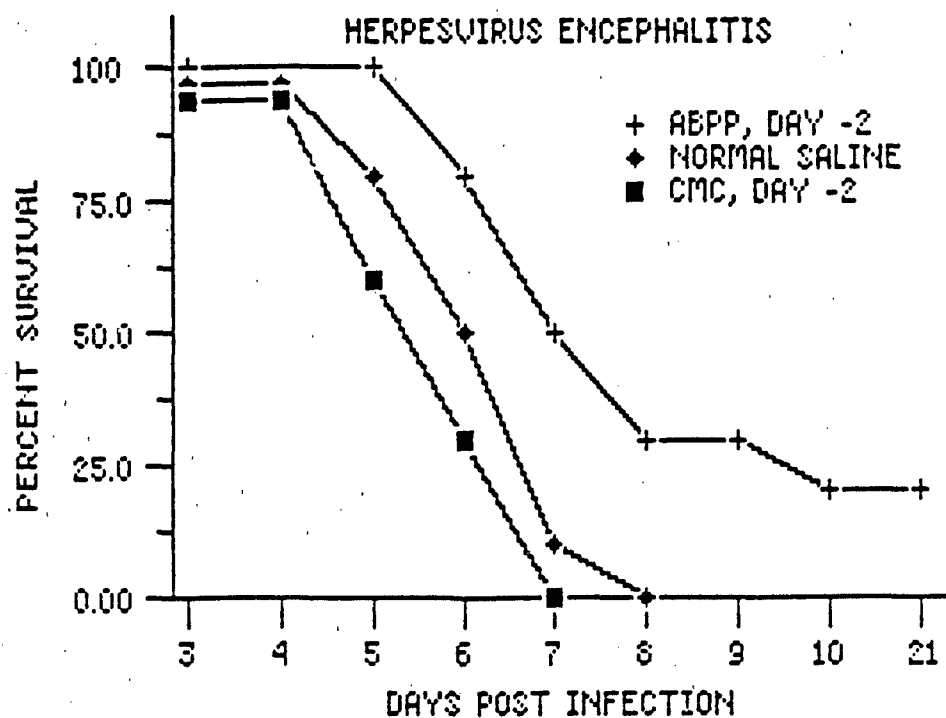
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.85	-
ACDFPP Day +1	8.03	NS
Saline Control	9.69	NS

Figure 41. Effect of ACDFPP, given on day +1, on resistance to herpesvirus-induced hepatitis.

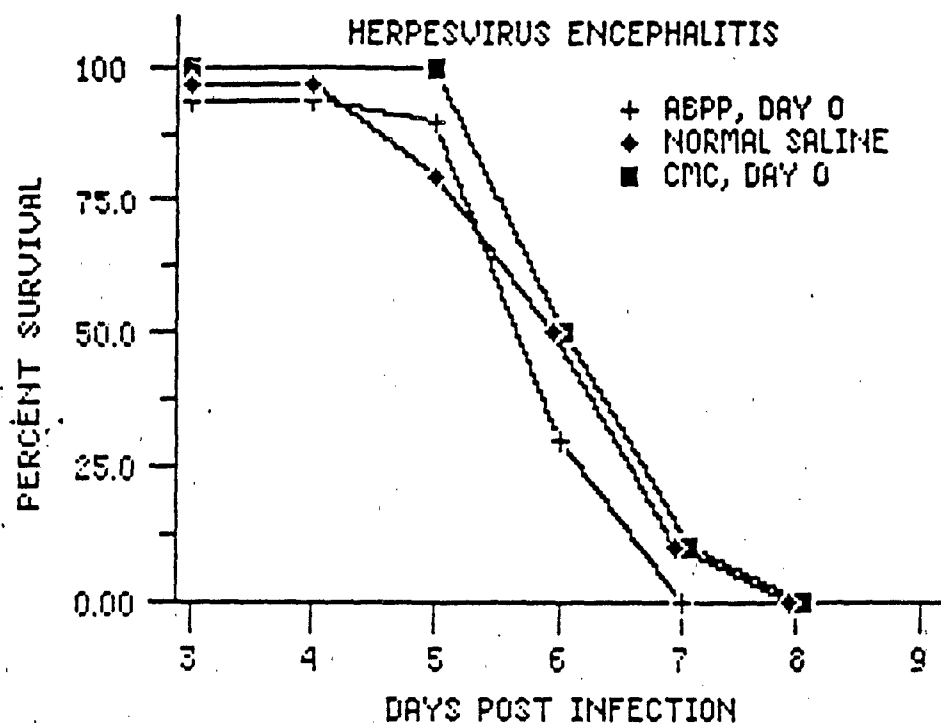
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intravenous challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.84	-
ABPP Day -2	7.82	<0.01
Saline Control	6.33	NS

Figure 42. Effect of ABPP, given on day -2, on resistance to herpesvirus-induced encephalitis.

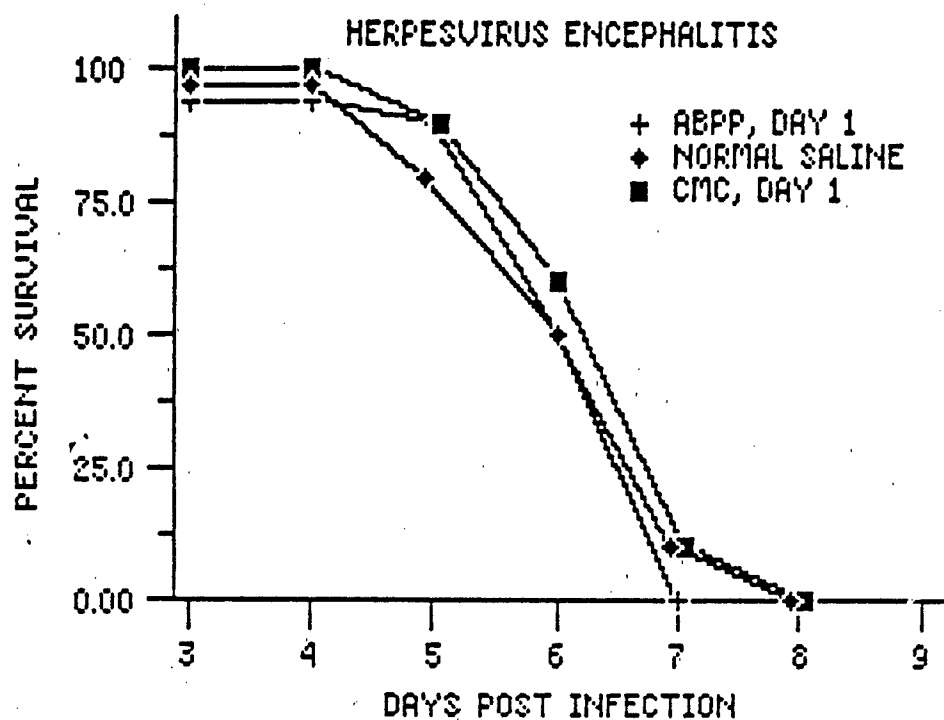
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.57	-
ABPP Day 0	6.17	NS
Saline Control	6.33	NS

Figure 13. Effect of ABPP, given on day 0, on resistance to herpesvirus-induced encephalitis.

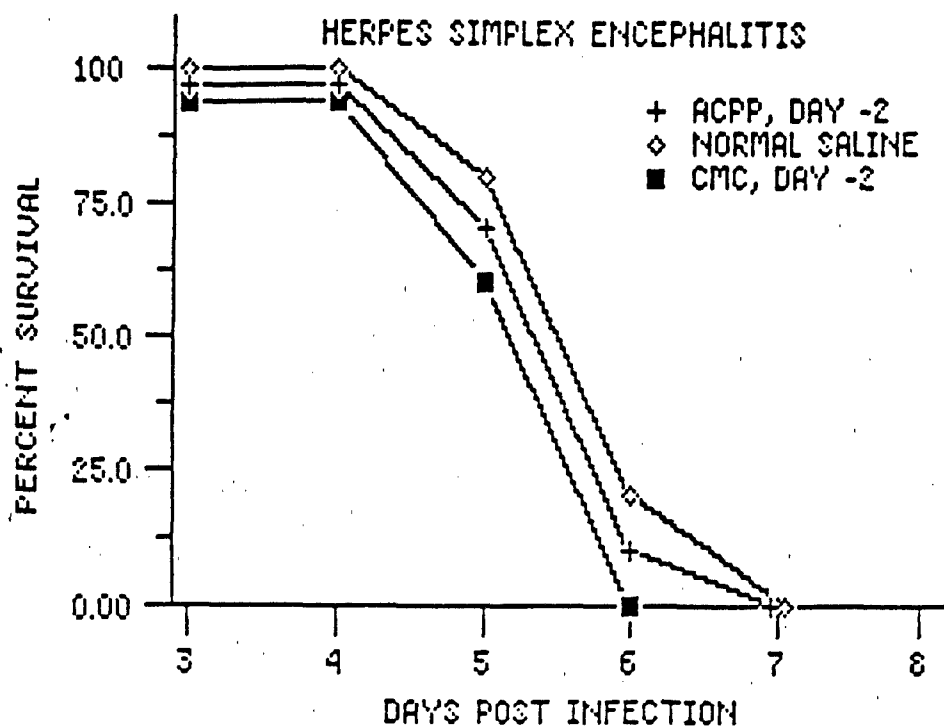
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.55	-
ABPP Day +1	6.36	NS
Saline Control	6.33	NS

Figure 44. Effect of ABPP, given on day +1, on resistance to herpesvirus-induced encephalitis.

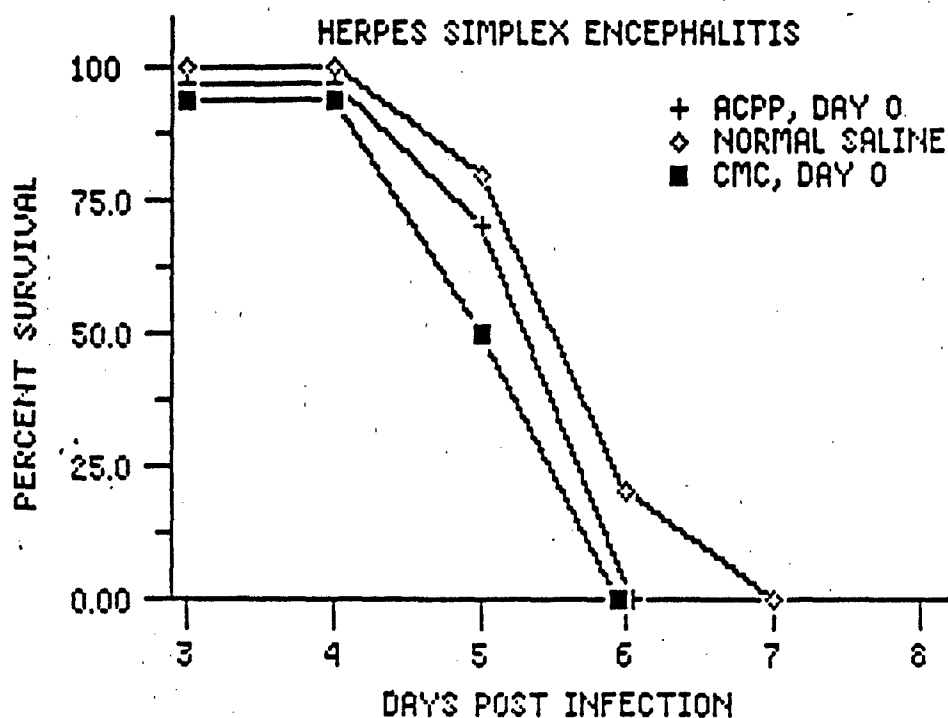
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.58	-
ACPP Day -2	5.77	NS
Saline Control	5.97	NS

Figure 45. Effect of ACPP, given on day -2, on resistance to herpesvirus-induced encephalitis.

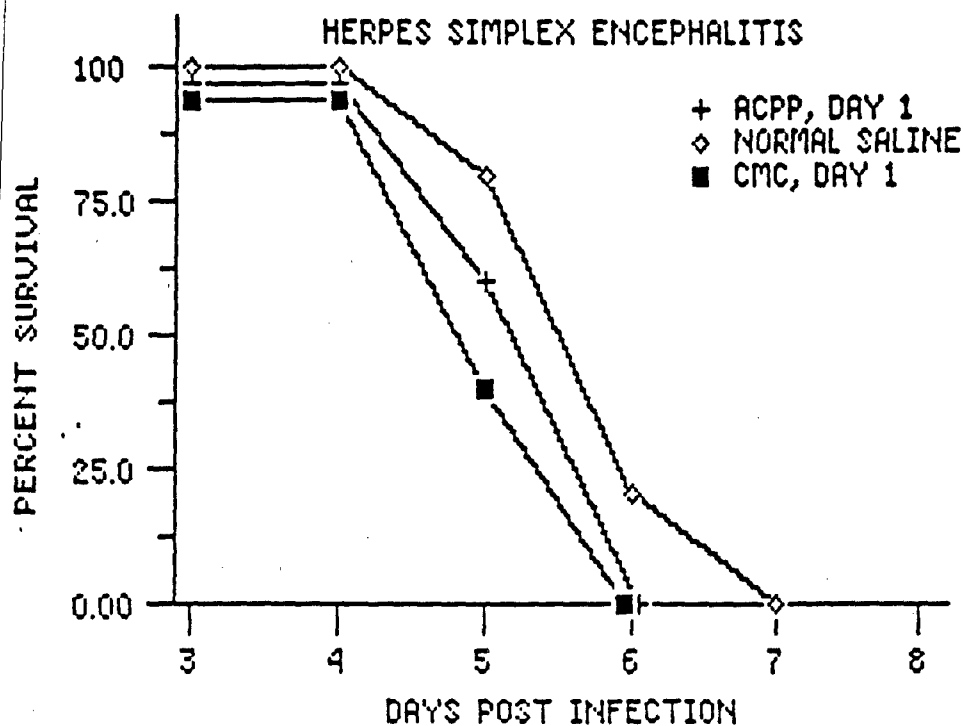
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.48	-
ACPP Day 0	5.68	NS
Saline Control	5.97	NS

Figure 46. Effect of ACPP, given on day 0, on resistance to herpesvirus-induced encephalitis.

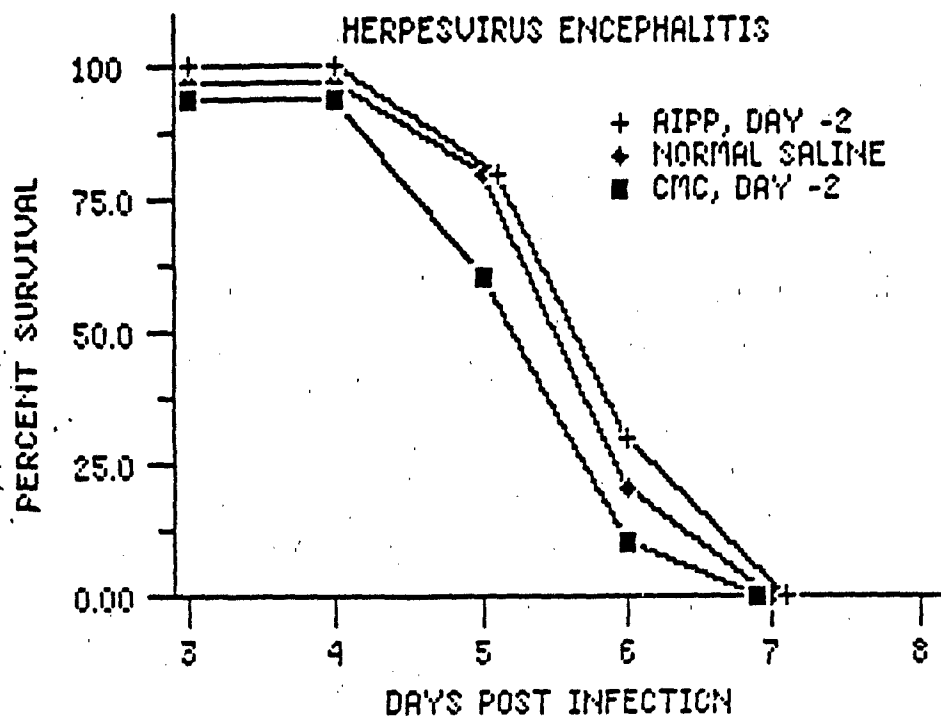
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.38	-
ACPP Day +1	5.58	NS
Saline Control	5.97	NS

Figure 47. Effect of ACPP, given on day +1, on resistance to herpesvirus-induced encephalitis.

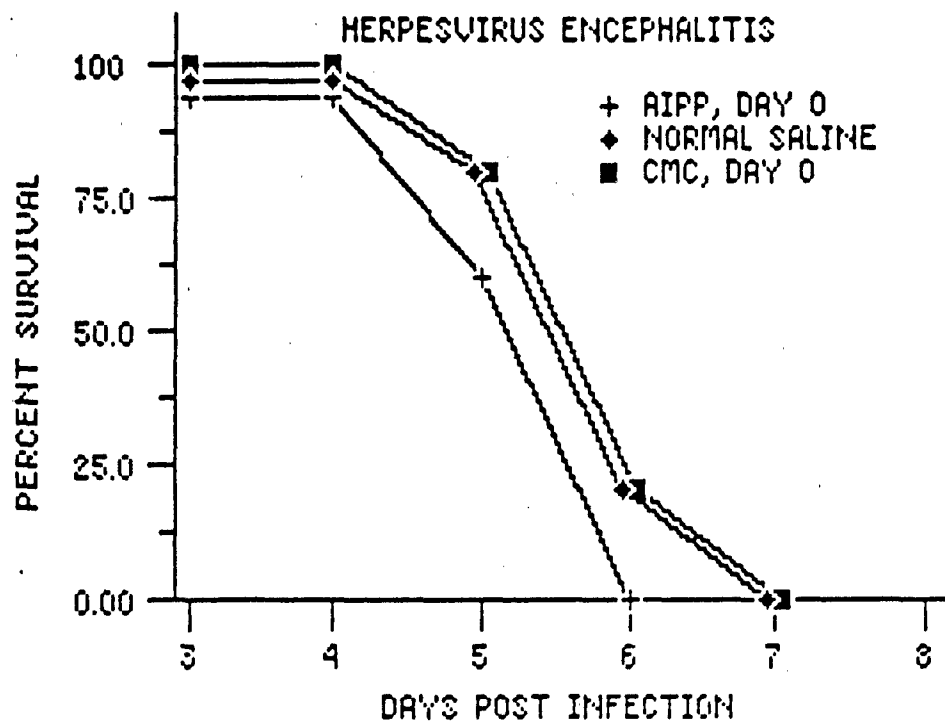
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.66	-
AIPP Day -2	6.06	NS
Saline Control	5.97	NS

Figure 48. Effect of AIPP, given on day -2, on resistance to herpesvirus-induced encephalitis.

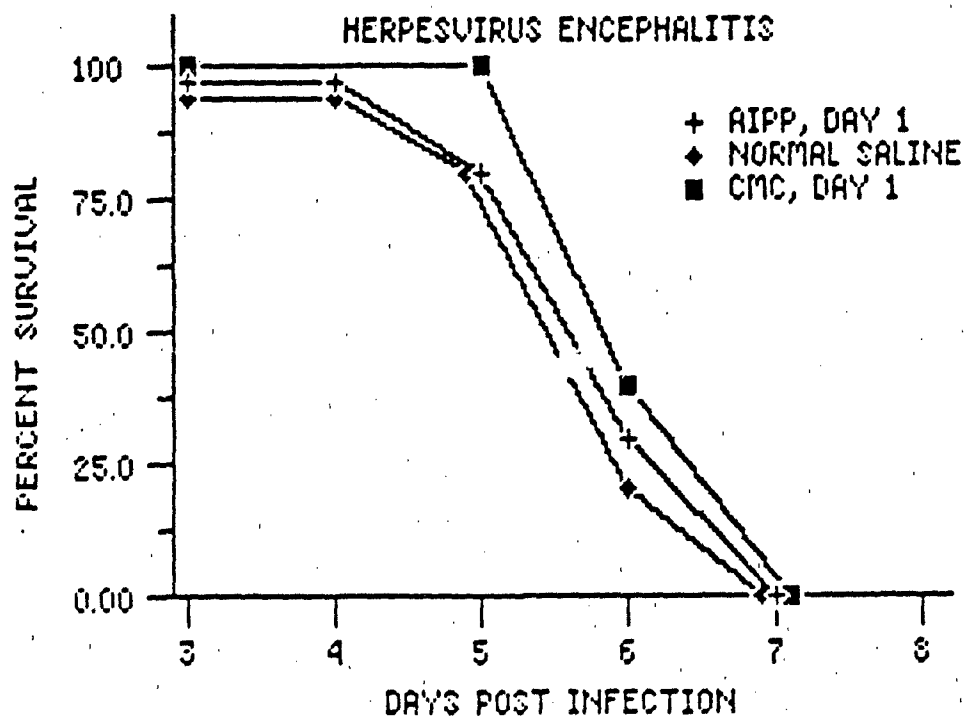
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.97	-
AIPP Day 0	5.58	NS
Saline Control	5.97	NS

Figure 49. Effect of AIPP, given on day 0, on resistance to herpesvirus-induced encephalitis.

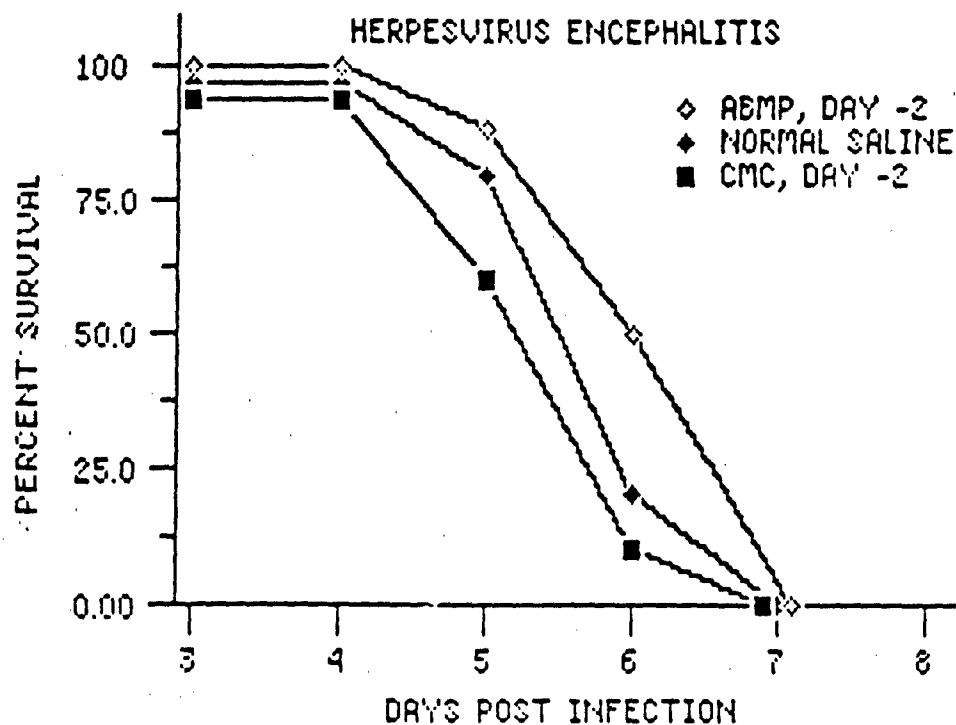
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.36	-
AIPP Day +1	6.06	NS
Saline Control	5.97	NS

Figure 50. Effect of AIPP, given on day +1, on resistance to herpesvirus-induced encephalitis.

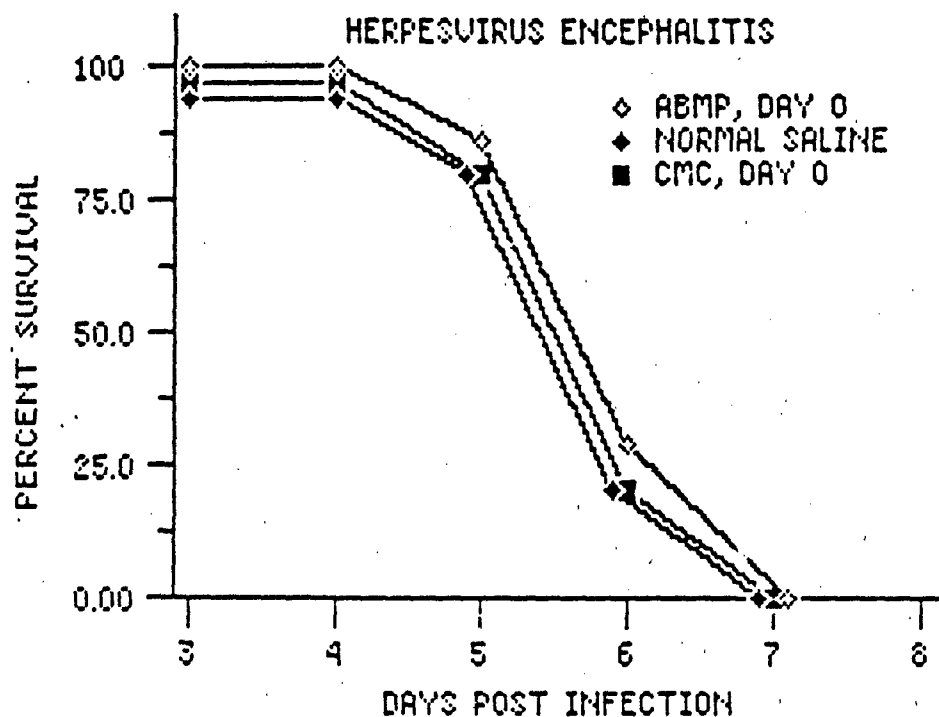
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) footpad challenge with $10^{5.0}$ of HSV-1 (MB Strain). Control animal received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.66	-
ABMP Day -2	6.30	NS
Saline Control	5.97	NS

Figure 51. Effect of ABMP, given on day -2, on resistance to herpesvirus-induced encephalitis.

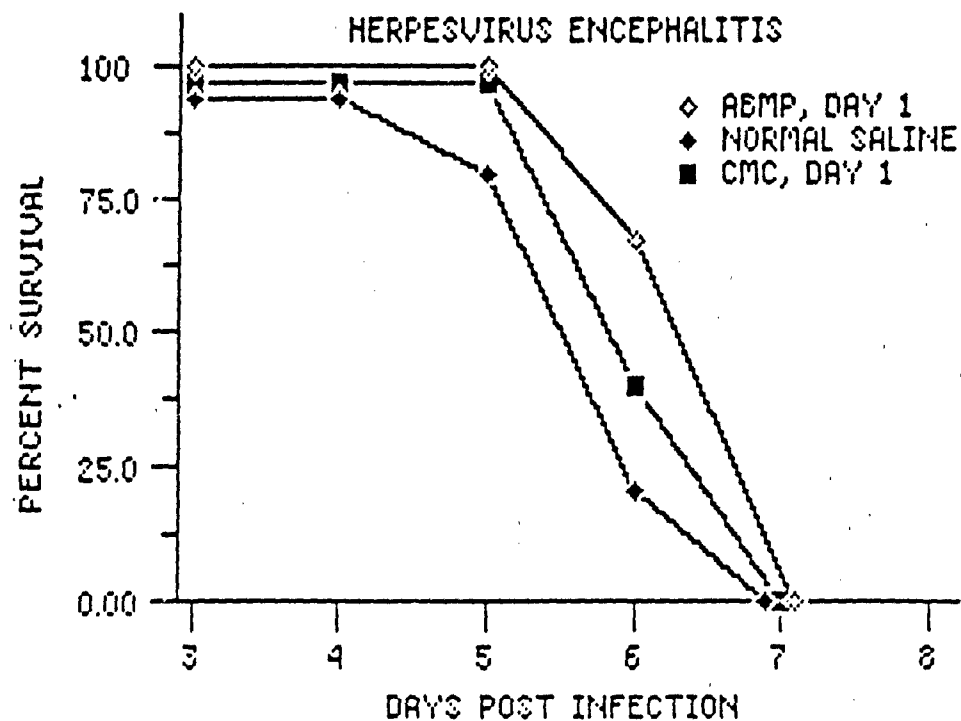
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.97	-
ABMP Day 0	6.11	NS
Saline Control	5.97	NS

Figure 52. Effect of ABMP, given on day 0, on resistance to herpesvirus-induced encephalitis.

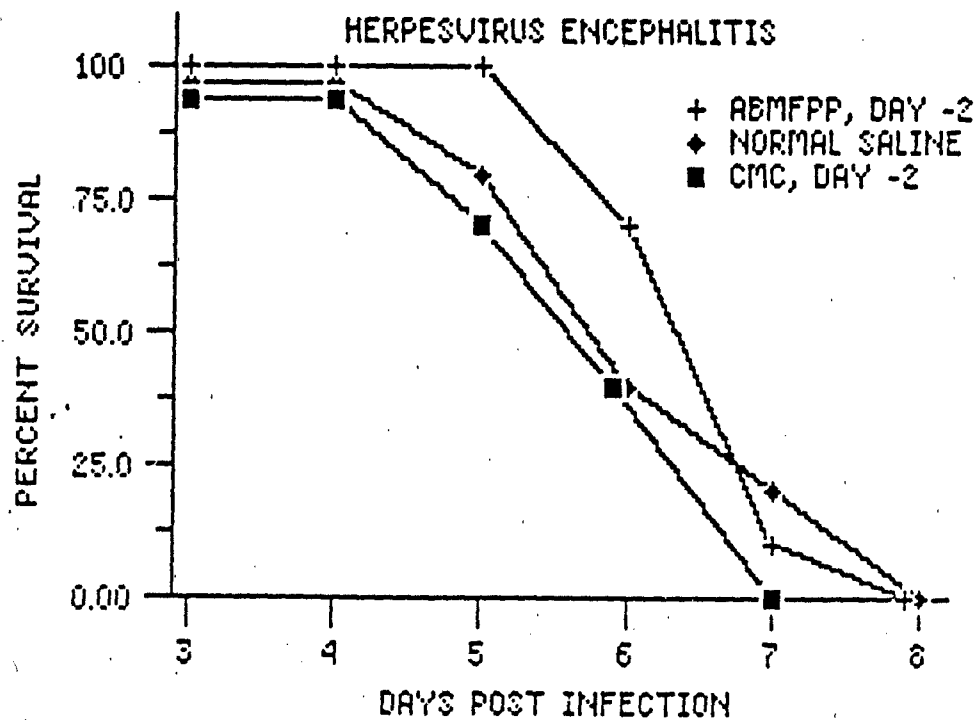
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.38	-
ABMP Day +1	6.32	NS
Saline Control	5.97	NS

Figure 53. Effect of ABMP, given on day +1, on resistance to herpesvirus-induced encephalitis.

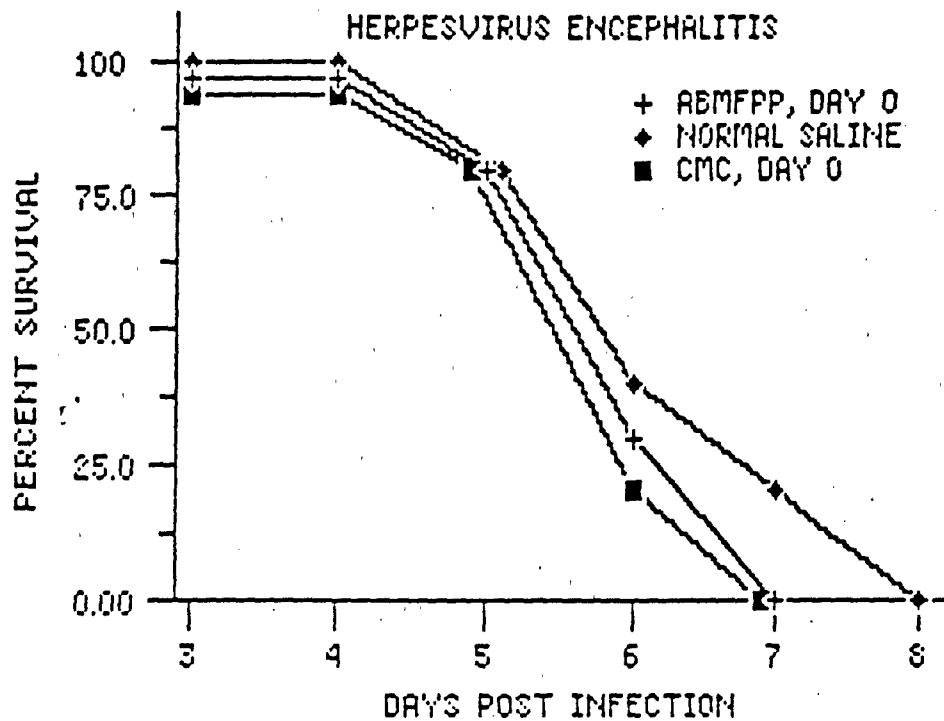
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.04	-
ABMFPP Day -2	6.77	NS
Saline Control	6.32	NS

Figure 54. Effect of ABMFPP, given on day -2, on resistance to herpesvirus-induced encephalitis.

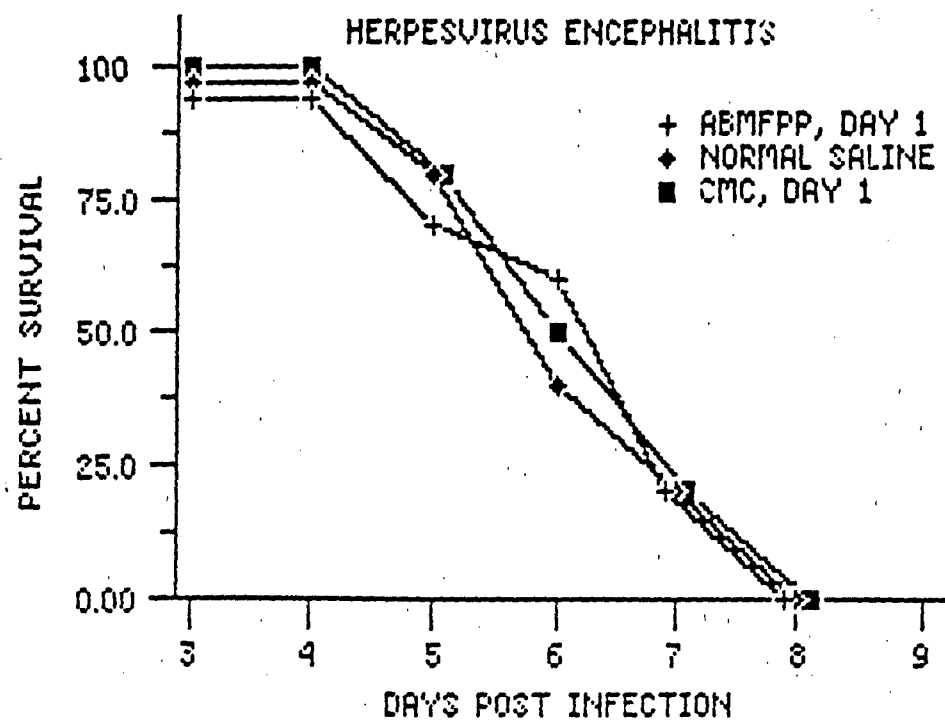
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.97	-
ABMFPP Day 0	6.06	NS
Saline Control	6.32	NS

Figure 55. Effect of ABMFPP, given on day 0, on resistance to herpesvirus-induced encephalitis.

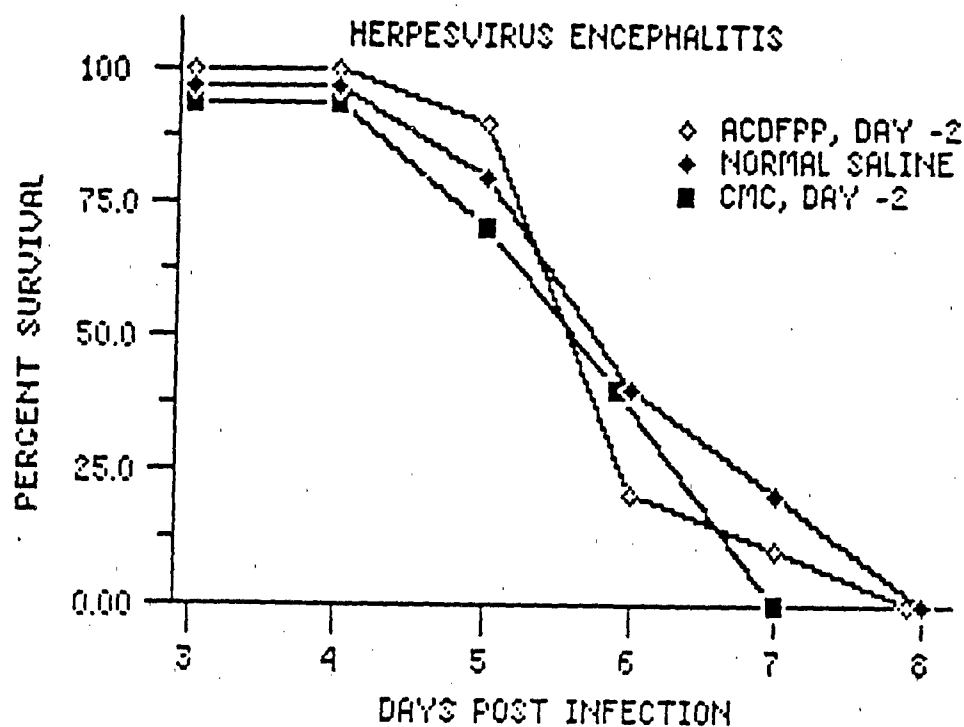
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.42	-
ABMFPP Day +1	6.40	NS
Saline Control	6.32	NS

Figure 56. Effect of ABMFPP, given on day +1, on resistance to herpesvirus-induced encephalitis.

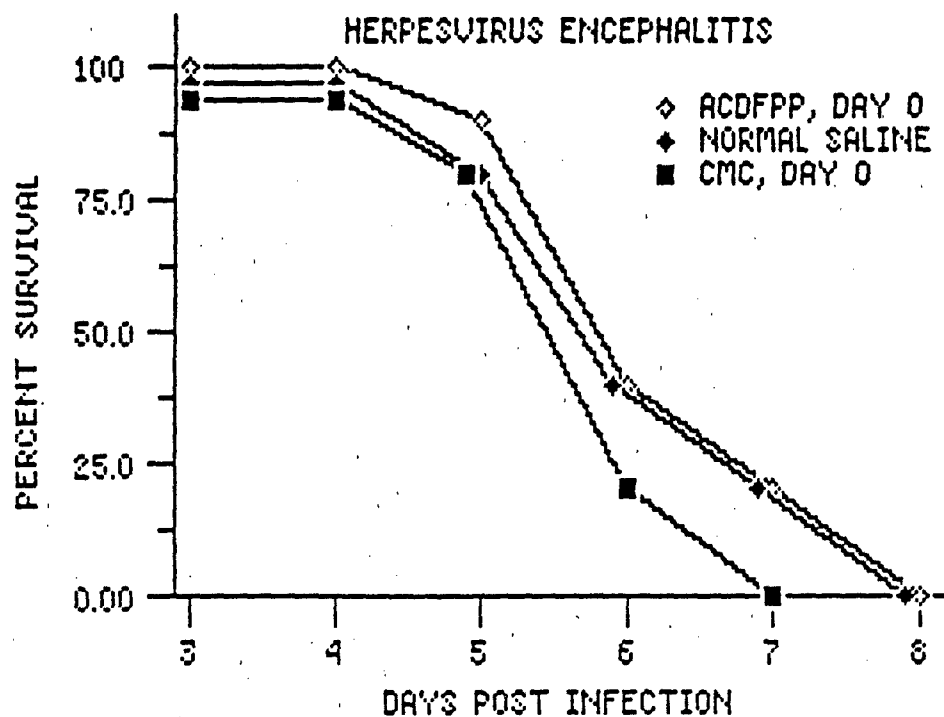
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one d after (D +1) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.04	-
ACDFPP Day -2	6.16	NS
Saline Control	6.32	NS

Figure 57. Effect of ACDFP, given on day -2, on resistance to herpesvirus-induced encephalitis.

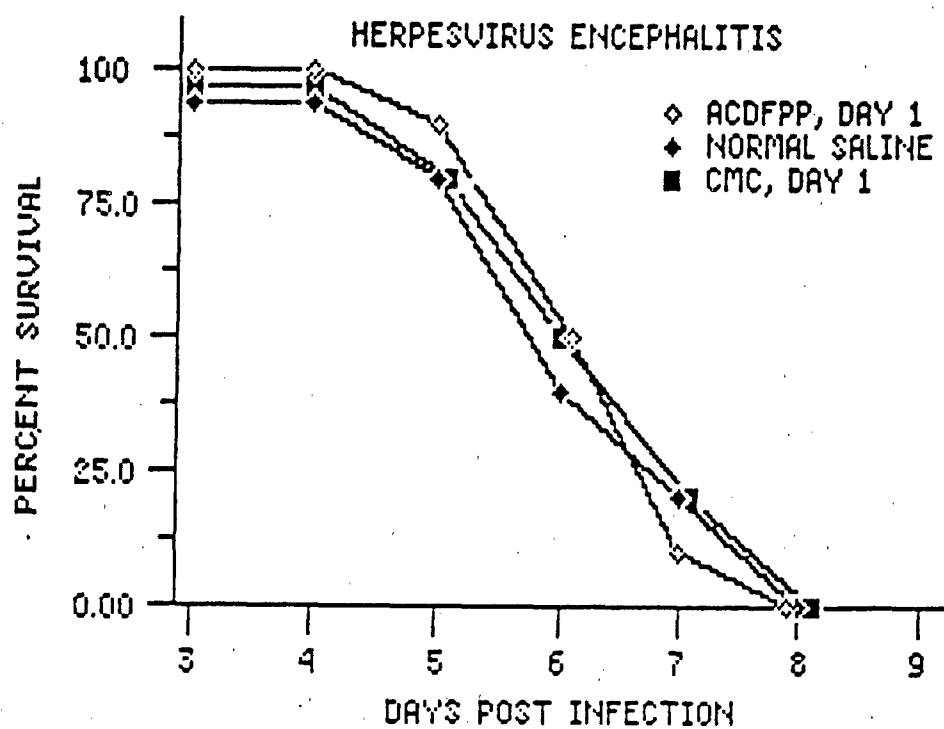
Mice were given ACDFP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.97	-
ACDFPP Day 0	6.44	NS
Saline Control	6.32	NS

Figure 58. Effect of ACDFPP, given on day 0, on resistance to herpesvirus-induced encephalitis.

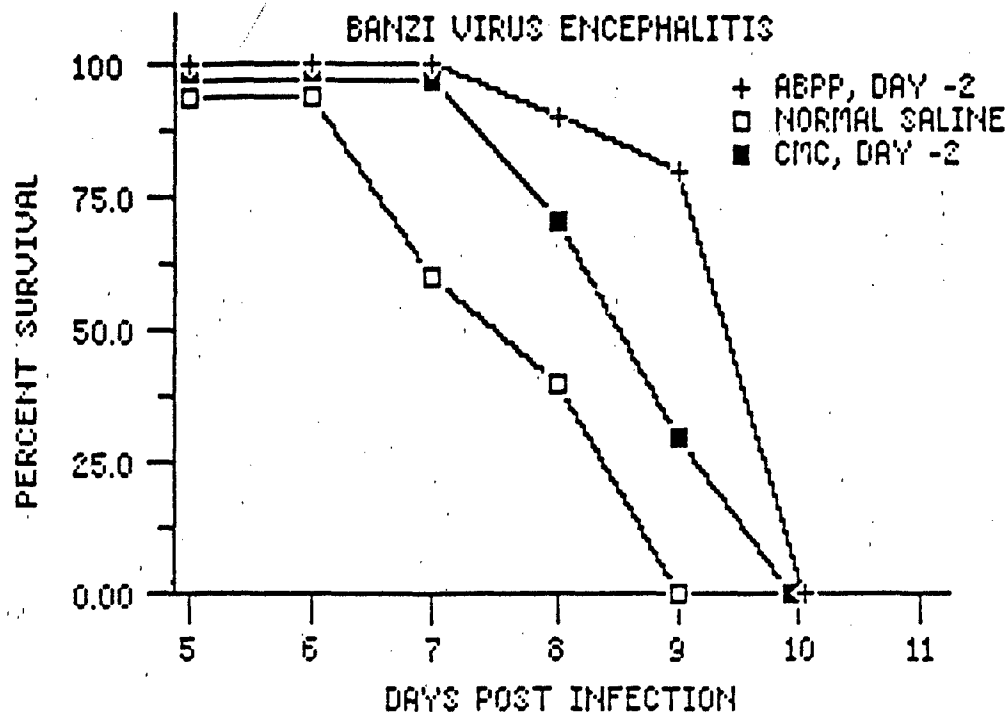
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.42	-
ACDFPP Day +1	6.45	NS
Saline Control	6.32	NS

Figure 59. Effect of ACDFP, given on day +1, on resistance to herpesvirus-induced encephalitis.

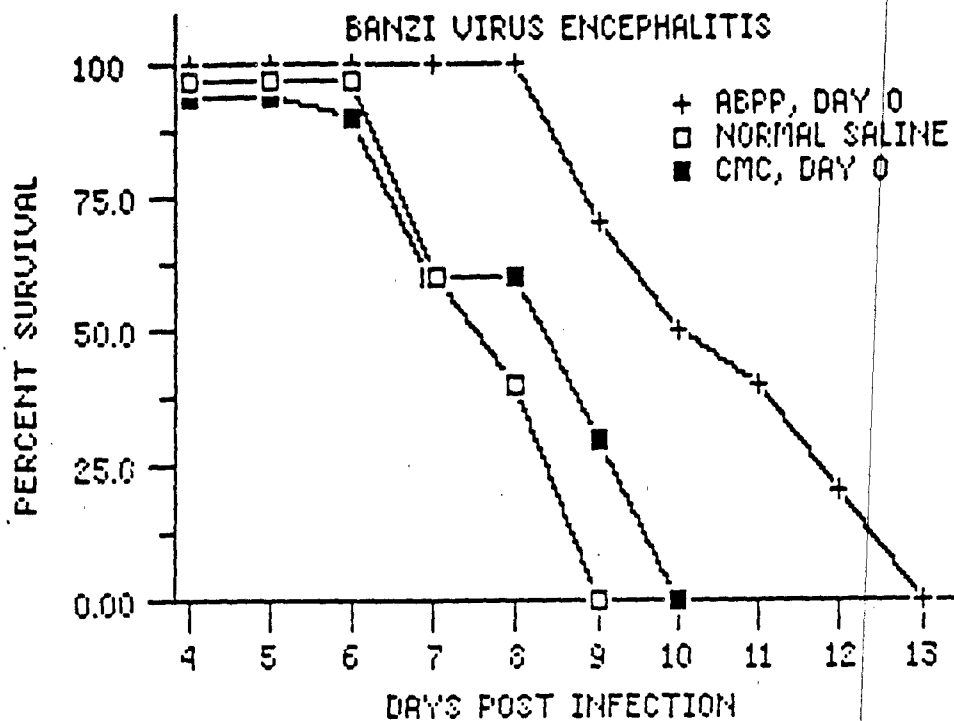
Mice were given ACDFP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) footpad challenge with 10 LD₅₀ of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.97	-
ABPP Day -2	9.68	NS
Saline Control	7.95	<0.025

Figure 60. Effect of ABPP, given on day -2, on resistance to banzivirus-induced encephalitis.

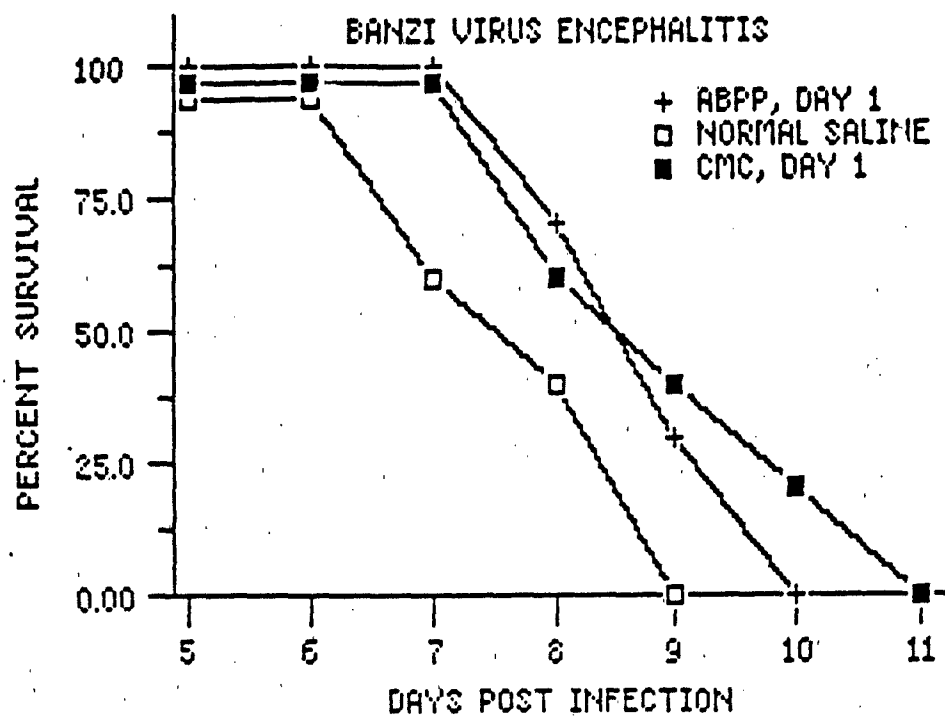
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.27	-
ABPP Day 0	10.69	<0.005
Saline Control	7.95	NS

Figure 61. Effect of ABPP, given on day 0, on resistance to banzivirus-induced encephalitis.

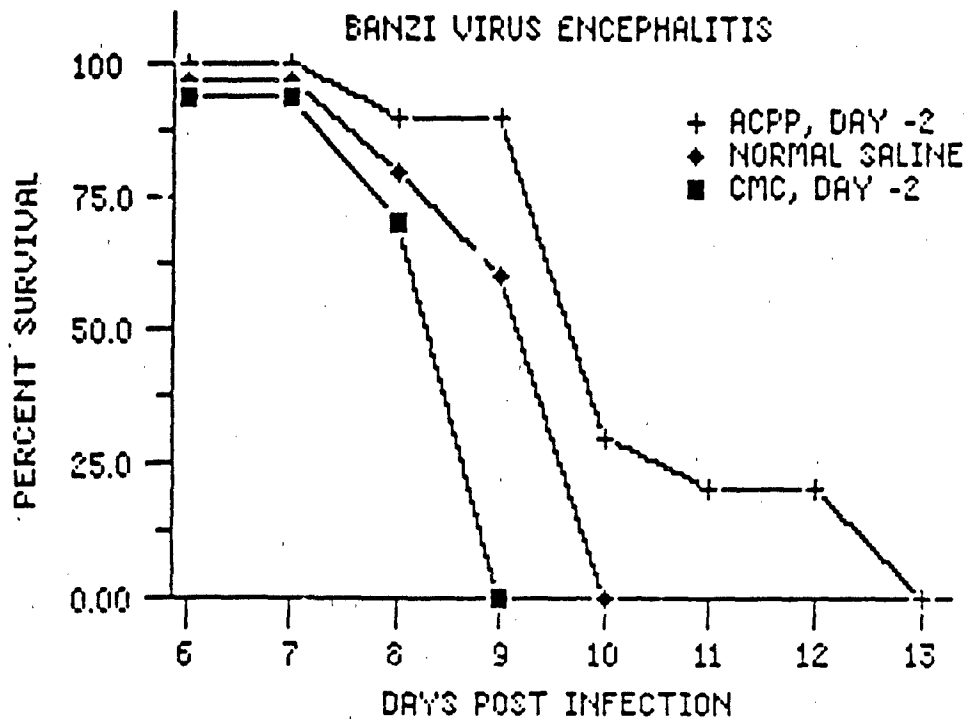
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	9.13	-
ABPP Day +1	8.97	NS
Saline Control	7.95	<0.025

Figure 62. Effect of ABPP, given on day +1, on resistance to banzivirus-induced encephalitis.

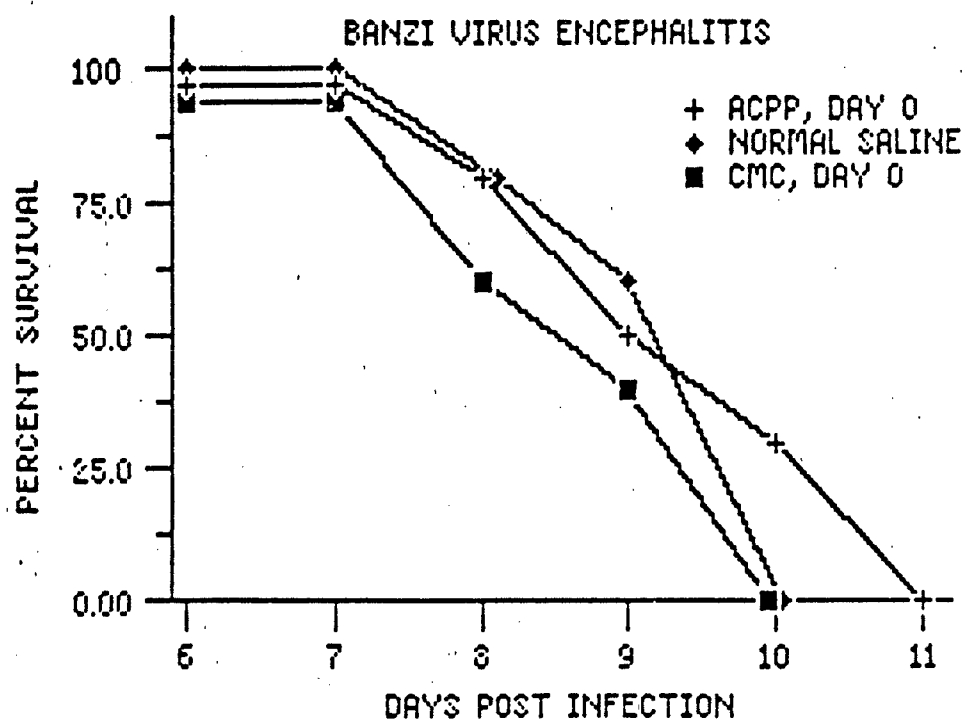
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.69	-
ACPP Day -2	10.40	<0.005
Saline Control	9.36	<0.05

Figure 63. Effect of ACPP, given on day -2, on resistance to banzivirus-induced encephalitis.

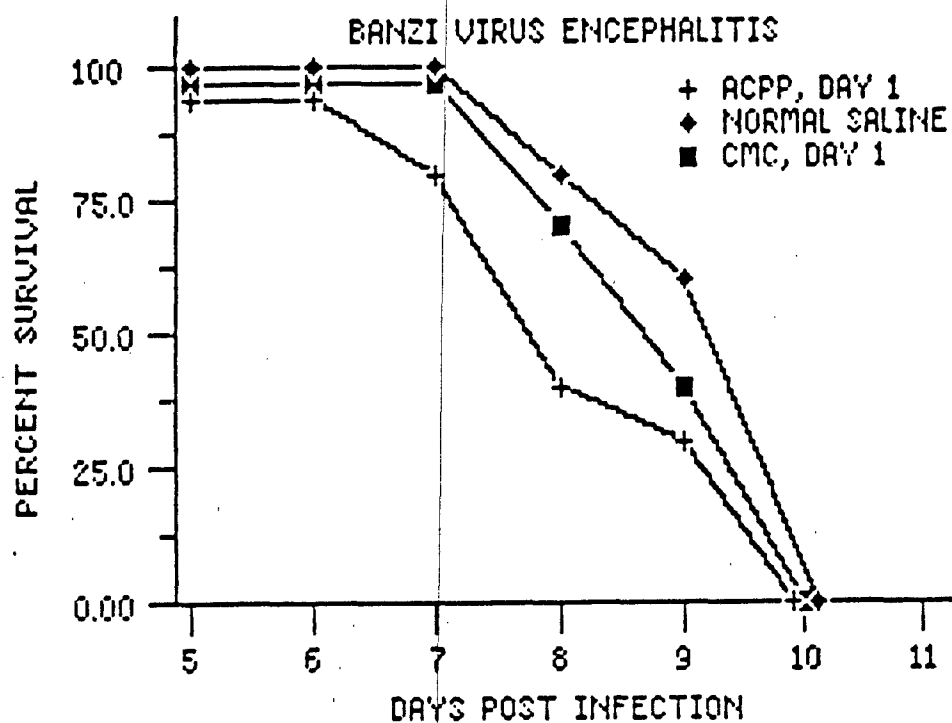
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.96	-
ACPP Day 0	9.53	NS
Saline Control	9.36	NS

Figure 64. Effect of ACPP, given on day 0, on resistance to banzivirus-induced encephalitis.

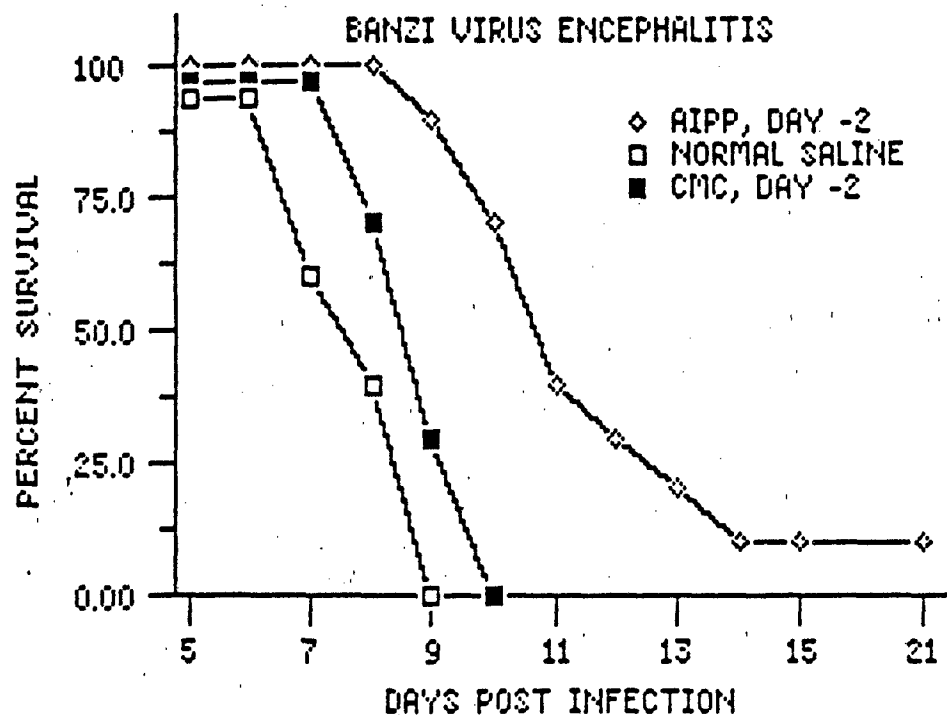
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	9.06	-
ACPP Day +1	8.43	NS
Saline Control	9.36	NS

Figure 65. Effect of ACPP, given on day +1, on resistance to banzivirus-induced encephalitis.

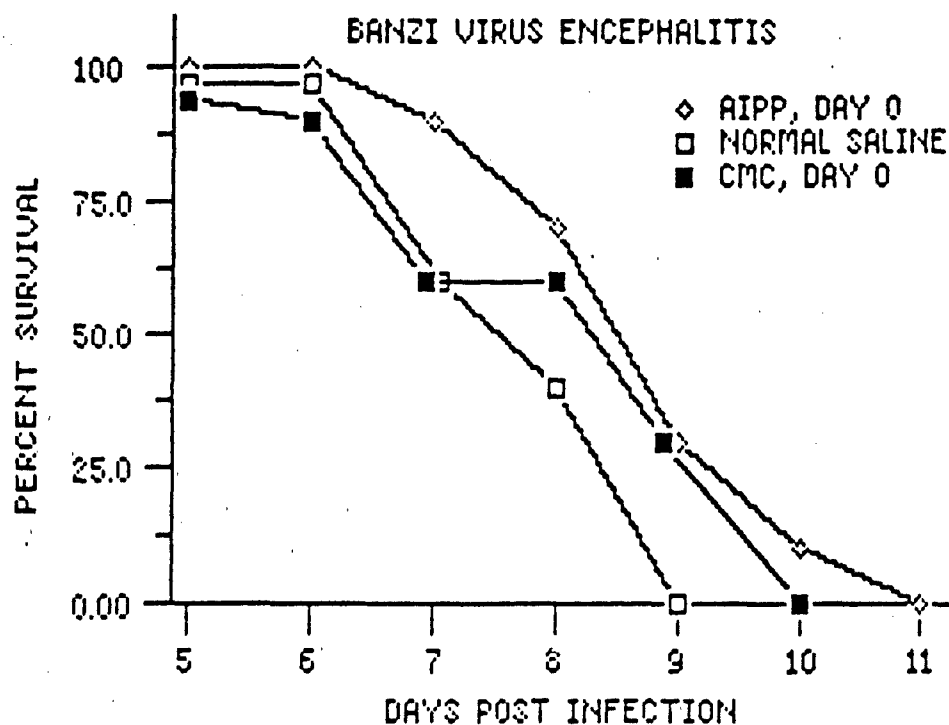
Mice were given ACPP* (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.97	-
AIPP Day -2	11.13	<0.001
Saline Control	7.95	<0.025

Figure 66. Effect of AIPP, given on day -2, on resistance to banzivirus-induced encephalitis.

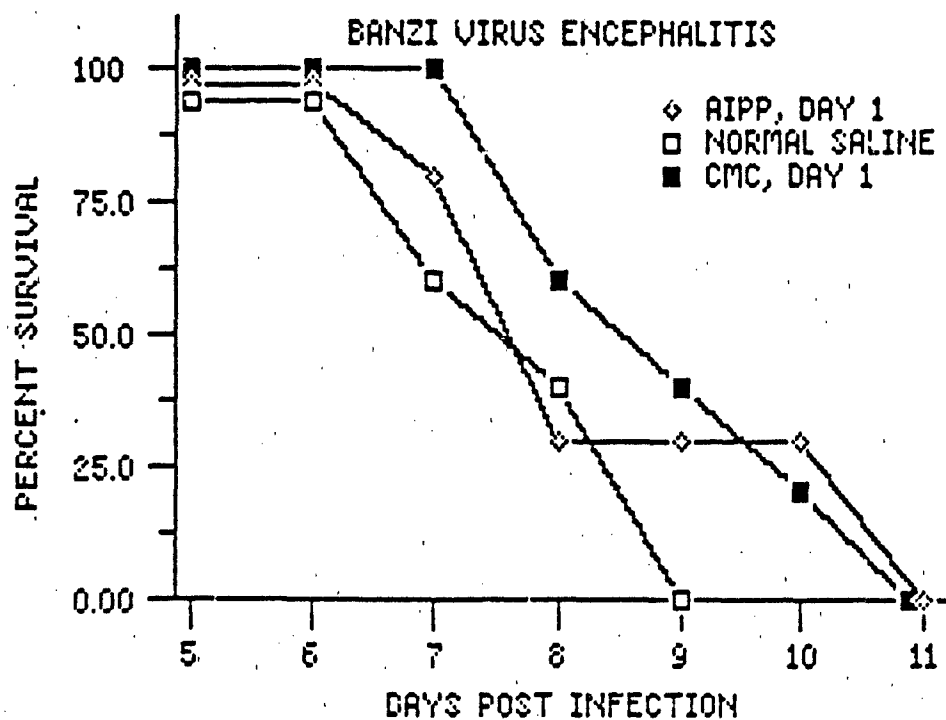
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.27	-
AIPP Day 0	8.93	NS
Saline Control	7.95	NS

Figure 67. Effect of AIPP, given on day 0, on resistance to banzivirus-induced encephalitis.

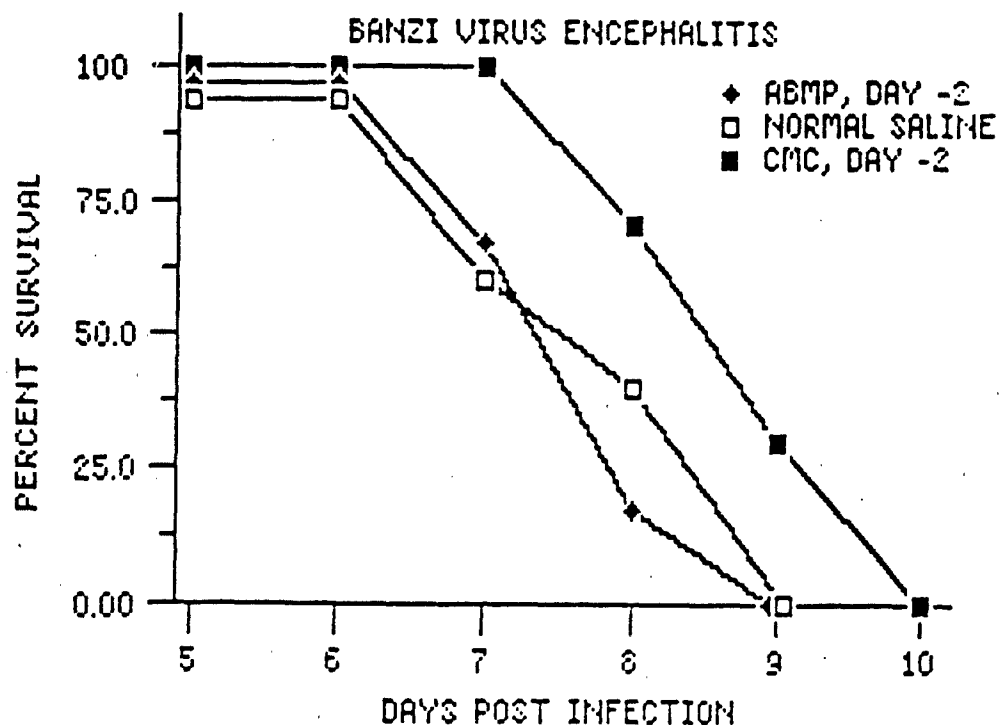
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	9.13	-
AIPP Day +1	8.57	NS
Saline Control	7.95	<0.025

Figure 68. Effect of AIPP, given on day +1, on resistance to banzivirus-induced encephalitis.

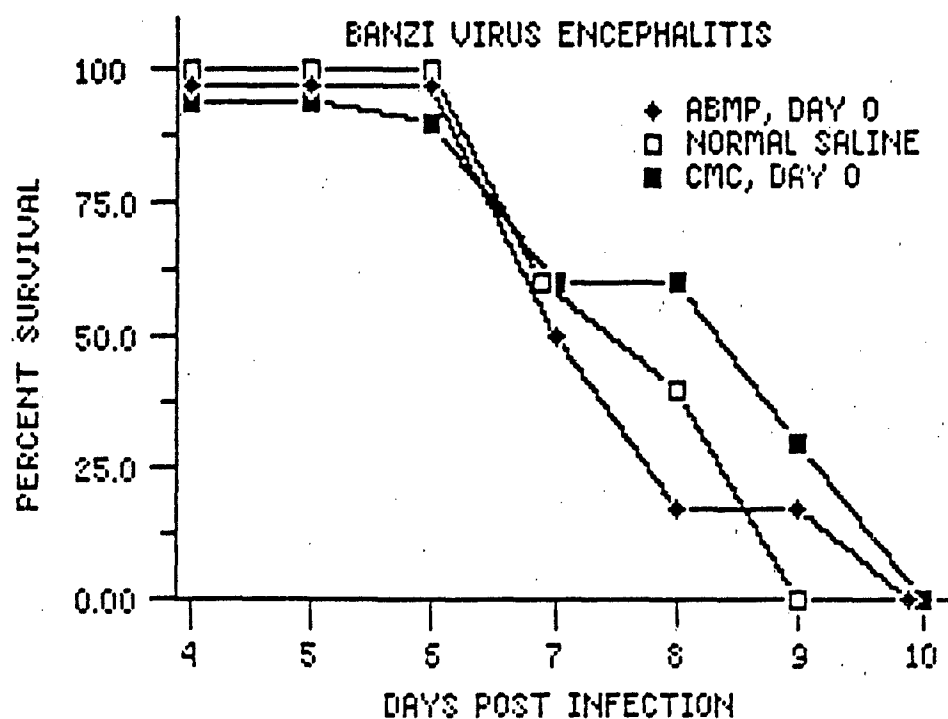
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.97	-
ABMP Day -2	7.80	<0.02
Saline Control	7.95	<0.025

Figure 69. Effect of ABMP, given on day -2, on resistance to banzivirus-induced encephalitis.

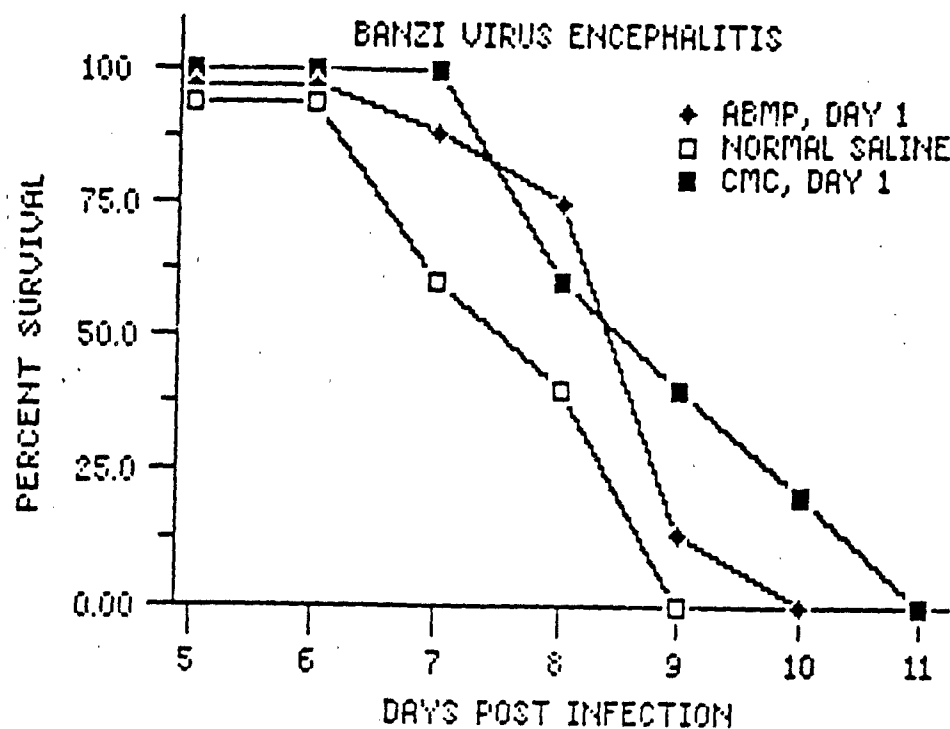
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.27	-
ABMP Day 0	7.77	NS
Saline Control	7.95	NS

Figure 70. Effect of ABMP, given on day 0, on resistance to banzivirus-induced encephalitis.

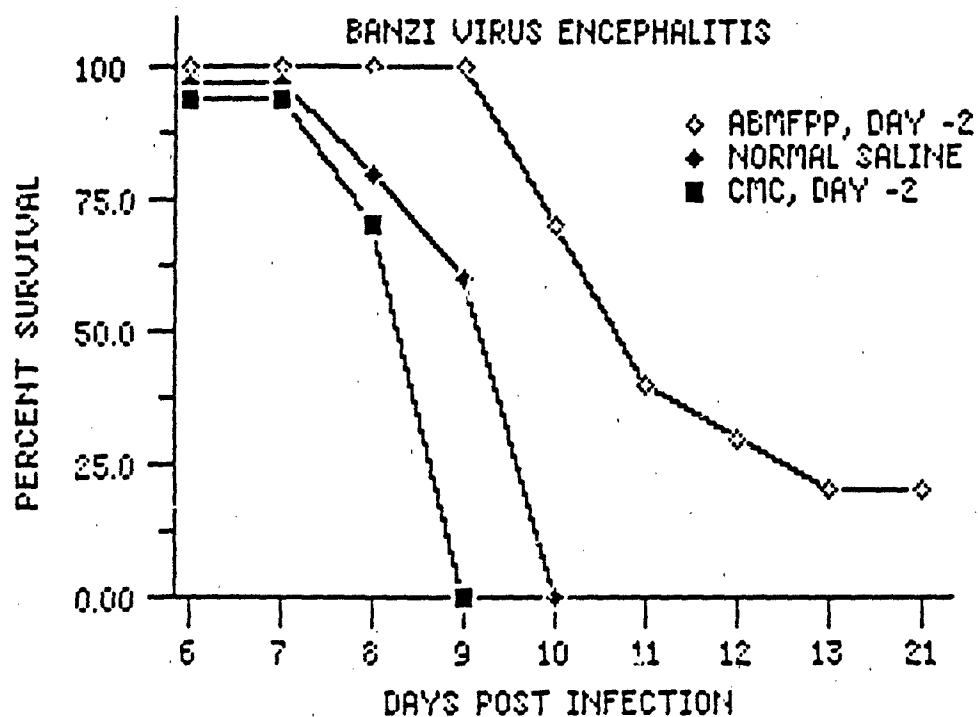
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	9.13	-
ABMP Day +1	8.71	NS
Saline Control	7.95	<0.025

Figure 71. Effect of ABMP, given on day +1, on resistance to banzivirus-induced encephalitis.

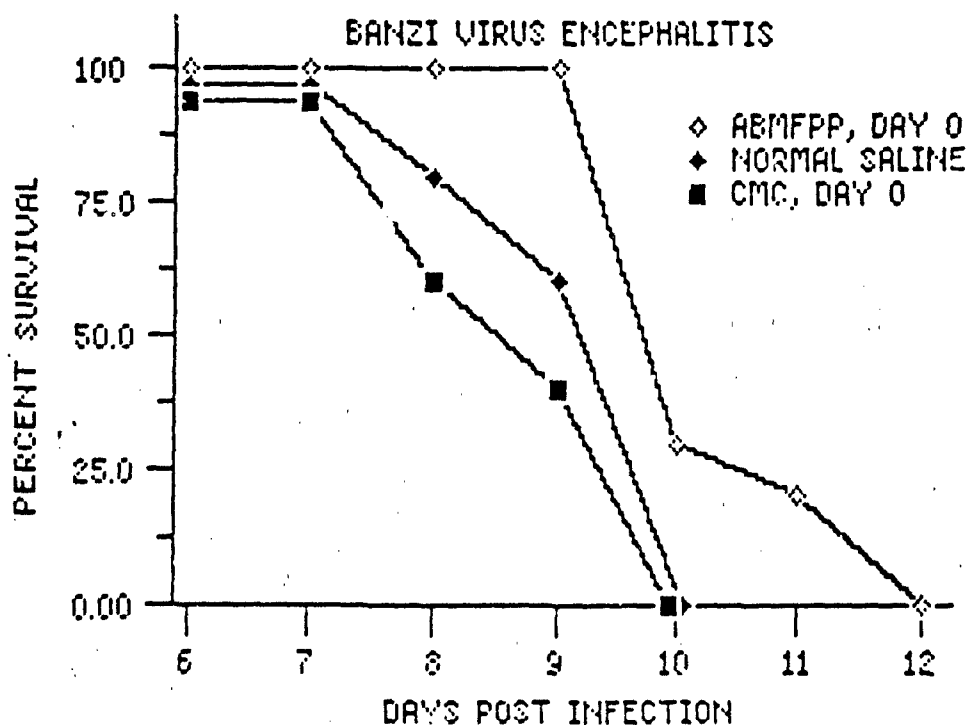
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.69	-
ABMFPP Day -2	10.96	<0.001
Saline Control	9.36	<0.05

Figure 72. Effect of ABMFPP, given on day -2, on resistance to banzivirus-induced encephalitis.

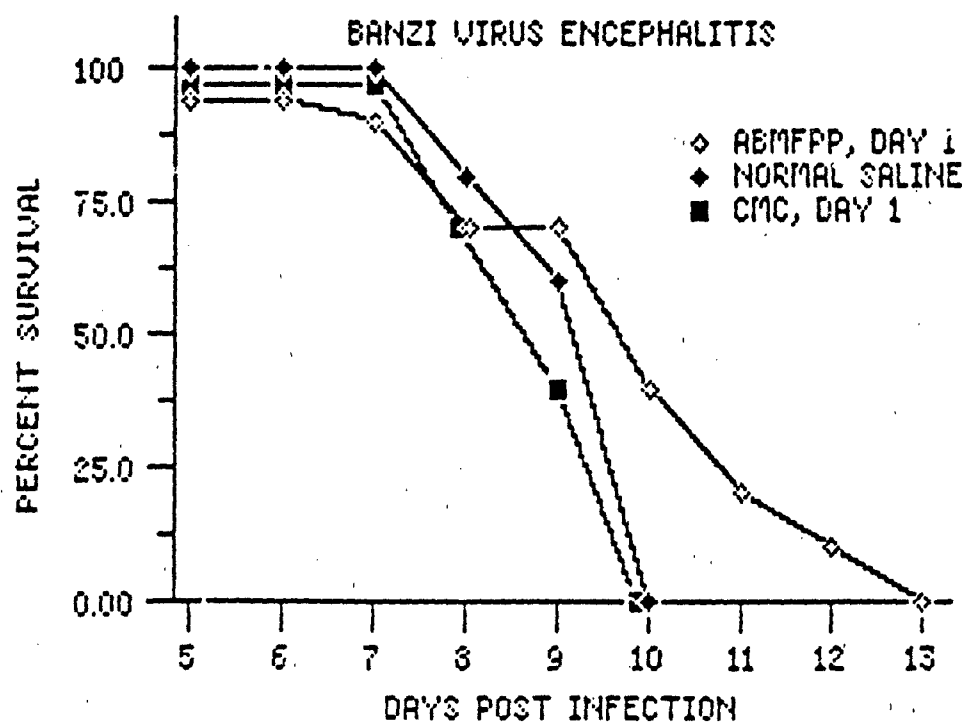
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.96	-
ABMFPP Day 0	10.47	<0.005
Saline Control	9.36	NS

Figure 73. Effect of ABMFPP, given on day 0, on resistance to banzivirus-induced encephalitis.

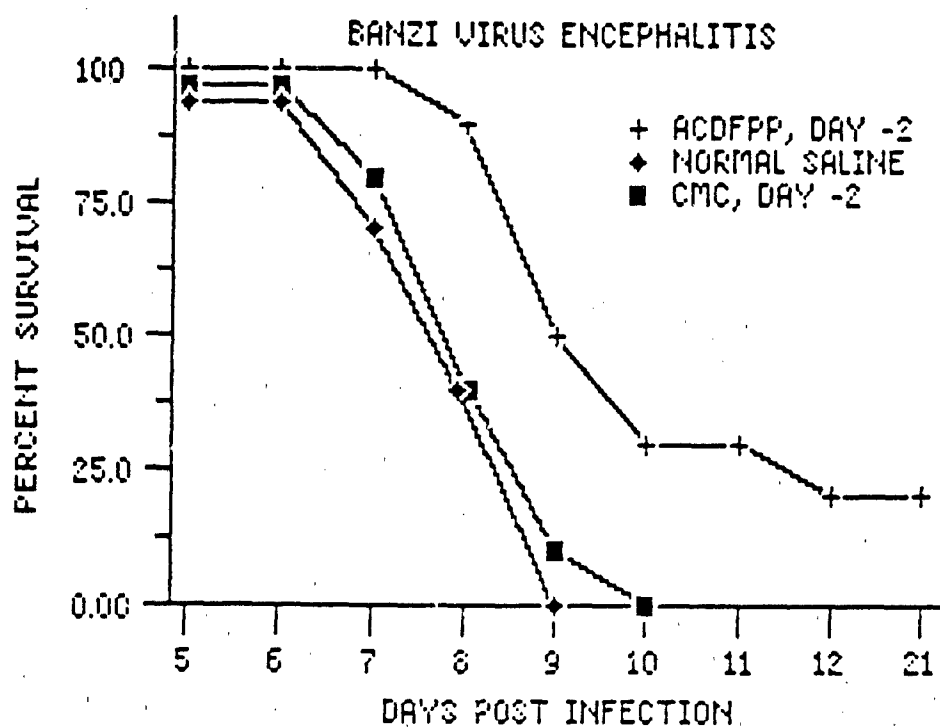
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	9.06	-
ABMFPP Day +1	9.83	NS
Saline Control	9.36	NS

Figure 74. Effect of ABMFPP, given on day +1, on resistance to banzivirus-induced encephalitis.

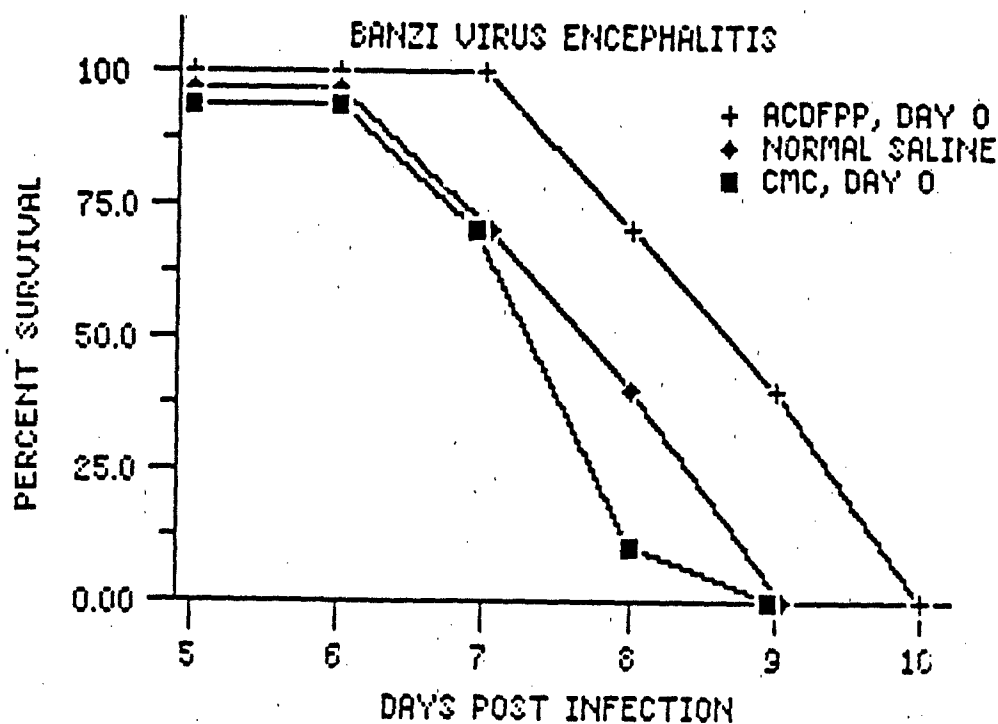
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.25	-
ACDFPP Day -2	9.44	<0.05
Saline Control	8.06	NS

Figure 75. Effect of ACDFPF, given on day -2, on resistance to banzivirus-induced encephalitis.

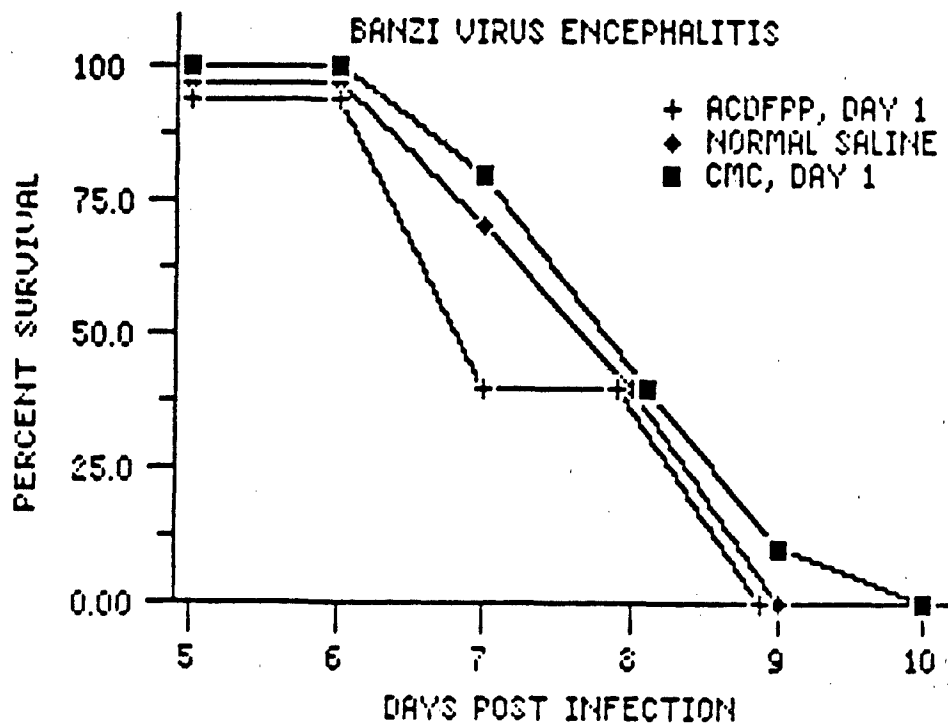
Mice were given ACDFPF (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.80	-
ACDFPP Day 0	9.07	<0.005
Saline Control	8.06	NS

Figure 76. Effect of ACDP, given on day 0, on resistance to banzivirus-induced encephalitis.

Mice were given ACDP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.25	-
ACDFFP Day +1	7.74	NS
Saline Control	8.06	NS

Figure 77. Effect of ACDFFP, given on day +1, on resistance to banzivirus-induced encephalitis.

Mice were given ACDFFP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) subcutaneous challenge with 10 LD₅₀ of banzivirus. Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.

DISTRIBUTION LIST

5 Copies

Commander
US Army Medical Research Institute of
Infectious Diseases
ATTN: SGRD-UIZ-M
Fort Detrick, Fredrick, MD 21701-5011

1 Copy

Commander
US Army Medical Research and Development Command
ATTN: SGRD-RMI-S
Fort Detrick, Fredrick, MD 21701-5012

12 Copies

Defense Technical Information Center (DTIC)
ATTN: DTIC-DDAC
Cameron Station
Alexandria, VA 22304-6145

1 Copy

Dean
School of Medicine
Uniformed Services University of the
Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814-4799

1 Copy

Commandant
Academy of Health Sciences, US Army
ATTN: ASH-CDM
Fort Sam Houston, TX 78234-6100